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**THE ADDITIVE EFFECT OF MELENGESTROL ACETATE (MGA) PRIMING AND
SODIUM MONENSIN ON REPRODUCTIVE PERFORMANCE IN BEEF HEIFERS**

A Thesis

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science**

in

The School of Animal Sciences

by

**Jonathan Lance Roberts
B.S., Louisiana State University, 2000
D.V.M., Ross University School of Veterinary Medicine, 2008
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ABSTRACT

Breeding beef heifers to produce calves at 2 years of age could be a profitable management decision. The current practices have proved inefficient, which has justified further research in the area. The objective of this study was to examine the effects of repeated progestogen priming on the attainment of puberty by prepubertal heifers, to evaluate the potential additive effect of progestogen priming plus sodium monensin on the attainment of puberty by prepubertal heifers, to examine subsequent reproductive performance, including the response to synchronization of the estrous cycle, first-service pregnancy rates and overall pregnancy rates and to examine the effects of repeated progestogen priming on follicular dynamics of prepubertal beef heifers. Data were collected and analyzed for birth weight, adjusted weaning weight, postweaning gain/loss in weight and daily gain throughout the experiment. Also the average frame score, average breeding weight, average breeding body condition score, average change in body condition score, average number of animals that showed estrus behavior prior to the breeding season, average number of animals that responded to synchronization, average number of corpora lutea formed during the treatment periods, reproductive tract score at breeding as well as the change in reproductive tract score, follicle populations, change in ovary scores throughout the experiment, pregnancy percentage and the average fetal age at pregnancy determination were evaluated. In this study, treatment groups fed sodium monensin (Rumensin and MGA+Rumensin groups) had significant ($P<0.05$) advancements in fertility and maturational status when compared with Control animals. The MGA group did excel in follicle development when compared with the Control group, but more heifers became pregnant from artificial insemination combined with natural mating in the Rumensin groups than in the Control group. In conclusion, these experiments provide evidence for the positive effect of sodium monensin on puberty attainment and increased overall pregnancy rates when using artificial insemination in conjunction with natural mating. However, the additive effect of melengestrol acetate, if any, is yet to be defined.

CHAPTER I

INTRODUCTION

Management of replacement beef heifers involves decisions that affect future productivity of the entire cow herd. Breeding heifers to calve at 2 years of age can be a profitable management decision for beef producers. Historically, heifers were first mated at 2 years of age, but as beef production systems have become more intensified, more producers have begun to breed their heifers as yearlings to calve as 2 year-olds. Heifers that are mated and conceive early within their first restricted breeding season will continue to calve earlier and wean heavier calves throughout their lifetime than those who conceive later (Lesmeister et al., 1973). This could serve as valuable source of income for cattlemen that run an intensive cow-calf operation. Management is the most important tool in a cattleman's operation. Patterson (2000) stated that most components of fertility that influence calving and subsequent reproductive performance are not highly heritable and proposed that the majority of the factors that contribute to reproductive performance could be influenced by proper management practices (see Patterson et al., 1992a; Patterson et al., 1992b; Patterson et al., 2000). Possibly one of the most profitable traits in a cow-calf operation is reproductive performance. If a cow does not conceive in an efficient and timely manner, the value of that animal decreases to only carcass value.

Management of heifers to calve at 2 years of age will ultimately increase yearly revenue by increasing the value of the producer's product. By managing beef heifers to calve at 2 years of age, as well as early within a restricted breeding season, a producer will not only increase the value of his or her cow herd, but will increase the value of the calves, because they generally will be earlier maturing and heavier at slaughter. Lesmeister (1973) demonstrated that the original heifer group in their experiment had a statistically significant effect on the average daily gain of calves in subsequent breeding seasons. Heifers mated to conceive by 15 months of age will continue to calve early and produce heavier calves if managed accordingly.

According to the USDA Agricultural Marketing Service, the average live price for heifers and steers was roughly 80 dollars per 100 pounds (USDA, 2003). For example, a heifer calving at 2 years of age will theoretically have one more calf during her lifetime

than those that calve at 3 years of age. If a producer owned 300 cows and every heifer calved at 2 years of age, weaned a heavy calf, and produced an animal that eventually sold at 700 pounds, he or she would have earned an extra 560 dollars per head over animals that calved at 3 years of age for a total of 168,000 dollars. Also, calving early rather than late within a 60-day breeding season can also yield an increased profit. A heifer or steer weighing 700 pounds would return 560 dollars. At a marginal average daily gain (ADG) of 1 pound a day, the same animal born at the end of a 60-day calving interval would only return 512 dollars. That is roughly 50 dollars per head that is lost due to late calving. If a producer had 150 animals out of 300 that were born late in the calving season, he or she would take a loss of approximately 7,500 dollars per year if the number of late born calves remained the same. The subsequent breeding season is of major importance as well. As a calving two year old, the animal is not only expending energy for reproduction, but growth also. The two year old calving heifer has a much higher energy demand than its counterparts and must be fed on a higher plane of nutrition to retain proper body condition prior to the second breeding season. If the proper energy balance is not met prior to the second breeding season, conception will be delayed and the profitable characteristics of the animal will be lost (Patterson et al., 2000).

The current dogma for fertility in young animals is that the younger they are, the less fertile they are. This is true to an extent. Another way to look at the fertility in beef heifers is to look at maturity as a threshold. The threshold in beef heifers should be defined as puberty and the first fertile estrous cycle. It may be possible to lower this threshold with certain management techniques. Management practices are a valuable tool, but genetic knowledge and selection of animals should play a major role in some aspects of management. To obtain heifers that calve at 2 years of age, a cattle producer must lower that threshold to a level where the heifers will conceive prior to 15 months of age.

Selection and management programs to develop heifers have primarily focused on physiological processes that result in, and influence, the onset of puberty. In addition, due to lowered fertility of the pubertal estrus and second estrus, replacement heifers need to reach puberty 1 to 3 months before the age at which they are to be bred

so they have gone through at least two estrous cycles prior to breeding (Byerley et al., 1987a; Morrow et al., 1970). Exogenous progestogens can hasten the onset of puberty in heifers; however, they may only prove effective when used near the time of natural puberty onset (Anderson et al., 1996; Gonzalez-Padilla et al., 1975c). Further, the addition of sodium monensin to the diet has also been found to hasten the onset of puberty in beef heifers (McCartor et al., 1979). However, at the present there is limited data available on the effects of priming heifers with an oral progestogen (MGA) multiple times to enhance heifer development, or the potential additive effects of MGA priming plus sodium monensin on pubertal onset and subsequent reproductive performance.

The overall purposes of this study were (1) to examine the effects of repeated progestogen priming of prepubertal heifers on the attainment of puberty, (2) to evaluate the potential additive effect of melengestrol acetate (MGA) plus sodium monensin on attainment of puberty in beef heifers, (3) to examine subsequent reproductive performance, including the response to synchronization of estrus, first service conception and overall pregnancy rates, and (4) to examine the effects of repeated progestogen priming on follicular dynamics of prepubertal heifers.

CHAPTER II

LITERATURE REVIEW

Genetic, Environmental, and Management Factors Affecting Puberty

Although management is of major importance when determining the time of puberty in beef females, the genetic make-up of that animal can also have some influence. This is especially prominent when age at puberty is compared across breeds. Ferrell (1982) reported that mean age and weight at puberty, regardless of mature size or growth rate, was significantly affected by breed type. Due to the major differences among breeds in birth weight, growth rate, mature size, body composition, age at puberty, and milk production, careful consideration should be made in selection of replacement animal genotype (Cundiff, 1986; Patterson et al., 1992b).

Wiltbank et al. (1966) reported that Angus heifers attained puberty at a younger age than that of Hereford heifers, while later Laster et al. (1972) reported that significantly more heifers born to Angus dams reached puberty by 15 months of age than heifers born to Hereford dams. It is a general consensus within the literature that beef breeds with larger mature body weights and frame sizes are older and heavier at puberty than their counterparts that are faster growing, small to medium framed animals (Arije and Wiltbank, 1971; Simerl et al., 1991; Wolfe et al., 1990). In addition, animals selected for high milk production and crossbred animals are generally younger at puberty (Dow et al., 1982; Grass et al., 1982; Laster et al., 1972; Lesmeister et al., 1973; Patterson et al., 1992b; Simerl et al., 1991; Wolfe et al., 1990). Wiltbank et al. (1966) and Laster et al. (1972) had consistent findings, reporting the heterotic effect for crossbred heifers was approximately a 35-day to 40-day decrease in age at puberty. Laster et al. (1972) also reported that Jersey heifers reached puberty at a significantly younger age than all other dairy and beef breeds studied. This is consistent with the general rule, because Jersey cattle are a milk production breed, typically small framed, fast growing and have a low mature body weight.

Crossbred beef heifers are generally heavier and younger at puberty than purebred heifers, and the consistency of crossbred animals to out perform purebred animals reproductively is demonstrated in numerous studies (Dow et al., 1982; Lesmeister et al., 1973; Patterson et al., 1992b; Wolfe et al., 1990). Short et al. (1990)

also suggested an additive affect of crossbreeding. They noted that crossbreeding with other breeds that attain puberty at the same age or less can decrease age at puberty. To compliment the findings of Short et al. (1990), Lammoglia et al. (2000) reported that sire breed of crossbred heifers had a highly significant influence on the percentage of heifers reaching puberty before the breeding season, as well as, age at puberty for their entire study. There are not just breed differences in reproductive development but also differences between subspecies.

Animals with heavy Brahman or *Bos indicus* influence tend to lag in sexual development when compared with the *Bos taurus* subspecies. Plasse et al. (1968) reported that the average age for puberty for 83 Brahman heifers was 19.4 months. This average age at puberty is somewhat higher than previous reports for *Bos taurus* breeds of cattle (Arije and Wiltbank, 1971; Dow et al., 1982; Laster et al., 1972; Wolfe et al., 1990). Patterson et al. (1991) reported a 92% to 67% difference in animals attaining puberty before the start of the breeding season for Angus x Hereford heifers and Brahman x Hereford heifers, respectively. This information is consistent with a previous study by Bolton et al. (1987), which reported that pregnancy rates in $\frac{1}{2}$ Brahman and $\frac{1}{4}$ Brahman crossbred heifers decreased to 25% and 49% lower than other crossbred animals. Rodrigues et al. (1999) suggested that this lag in reproductive development in *Bos indicus* breeds was due to a difference in the endocrinology of puberty.

Within breeds, there seems to be differences in age at puberty. The smaller framed animals that gain weight faster tend to mature early. A predictor of these traits, as well as age at puberty regardless of weight gains, is sire scrotal circumference. Smith et al. (1989) reported that bulls with larger scrotal circumference tend to have offspring with moderate birth weight, above average growth rates and daughters that mature early. It has been reported for crossbred beef and dairy cattle that there is a correlation between sire scrotal circumference and age at puberty of his offspring (Martin et al., 1992; Smith et al., 1989). In contrast, Martinez-Velazquez et al. (2003) did not observe any strong correlations between sire scrotal circumference and age at puberty of beef cattle.

There has been little scientific literature suggesting that there is a correlation between age and puberty for cattle, but rather an attainment of a target weight prior to

puberty. Heifers must reach this target weight to attain puberty, which is a percentage of the mature body weight (Lamond, 1970; Patterson et al., 1992b; Patterson et al., 2000; Taylor and Fitzhugh, 1971). Beef heifers can be expected to reach puberty when they reach a genetic predetermined weight, which is approximately 65% of the mature weight of the animal (Lamond, 1970; Patterson et al., 1992b; Patterson et al., 2000; Taylor and Fitzhugh, 1971). Therefore, animals with the potential to achieve a greater mature body weight will have to meet a greater weight requirement to reach puberty in a timely manner. Only after this genetic predetermined weight is achieved can optimal pregnancy percentages for yearling heifers can be attained.

The ability of mature animals to hasten the onset of puberty through biostimulation has been studied somewhat in cattle and has conflicting results (Izard and Vandenberg, 1982; Taylor et al., 1992). Pheromones in urine, feces and cutaneous glands can elicit behavioral and endocrine responses via olfactory and hypothalamic systems, generating pulses of Gonadotropin Releasing Hormone (GnRH) (Rekwot et al., 2001). Izard and Vandenberg (1982) reported that there was a tendency for beef heifers, treated with urine from mature bulls, to attain puberty and calve earlier, suggesting that there is a priming pheromone in bull urine that can hasten the onset of puberty in beef heifers. Roberson et al. (1991) indicated that onset of puberty is hastened by the presence of a mature bull, but the effect was dependent on the growth rate of the animal. Small et al. (2000) also tested bull exposure in conjunction with the ionophore lasalocid on the attainment of puberty in beef heifers. They suggested that the ability of bull exposure and lasalocid treatment to hasten the onset of puberty in beef heifers was dependent upon the nearness of heifers to the attainment of puberty when the treatments were introduced. In contrast, weekly oronasal treatments with bull urine did not affect cyclicity, response to synchronization or pregnancy rates of crossbred beef heifers (Taylor et al., 1992). Wehrman et al. (1996) reported that placing a mature bull with prepubertal heifers did not induce precocious puberty. Roberson et al. (1987) also reported that biostimulation by a mature bull does not hasten onset of puberty nor weight at puberty of beef heifers, noting that ovarian size was also not influenced. Roberson et al. (1983) also noted that

placement of prepubertal beef heifers with estrous females did not decrease age at puberty.

Nutrition, Growth Rate, and Age at Puberty

It is generally considered that diet before puberty has the greatest influence on age at puberty in beef cattle. The importance of diet from birth to puberty has been studied extensively. Not all literature is in total agreement, but all agree on a number of items. Reproductive development is hastened by increased weight gains to a level, however negative affects are accrued by overfeeding (Arije and Wiltbank, 1971; Ferrell, 1982; Short and Bellows, 1971). Poor nutrition during the prepubertal period will delay puberty by inhibiting maturation of the endocrine system in beef cattle (Day et al., 1986). Weight gains in excess can result in reduced incidence of estrus, poor conception, high embryonic mortality and poor mammary development, while having a prolonged influence on the lifelong reproductive performance of beef and dairy heifers (Ferrell, 1982).

The conflicting elements of the scientific literature consist of timing of gain, average daily gains and body composition. Numerous reports in the scientific literature indicate any or all of these elements may have an effect on attainment of puberty in beef heifers. Earlier in the literature, it has been indicated that preweaning rate of growth can have a greater influence on puberty than gains during the postweaning period in beef and dairy heifers (Clanton et al., 1983; Dufour, 1975; Joubert, 1954; Little et al., 1981; Menge et al., 1960; Patterson et al., 1992b; Sorenson et al., 1954; Swierstra et al., 1977). Additionally, one report suggested that high rates of gains during the postweaning period delayed the onset puberty (Arije and Wiltbank, 1971). It has been reported that beef heifers exhibiting higher growth rates during the preweaning period reached puberty at a younger age and heavier body weight (Arije and Wiltbank, 1971; Brown et al., 1972; Laster et al., 1972; Swierstra et al., 1977; Wiltbank et al., 1966).

In contrast, postweaning average daily gains has also been indicated to have a high correlation to age at puberty. Dunn et al. (1969) reported that postweaning growth rate was highly associated with age and body weight at puberty. In a later study, Short and Bellows (1971) reported that when the postweaning rate of gain was increased from

0.45 kg/day to 0.68 kg/day, age at puberty decreased from 411 days to 380 days. They also noted that heifers fed to gain 0.23 kg/day during the postweaning phase had a decreased occurrence of estrus, lower pregnancy rate, higher pregnancy loss and greater time to conception than their counterparts. Granger et al. (1989) also suggested that as the level of nutrition increased, age at puberty decreased. One year later, Granger et al. (1990) supported their previous findings, reporting that age at puberty decreased with increased dietary components and weight loss during the postweaning period had adverse effects on reproductive development. Hall et al. (1994) also concluded that beef heifers fed a diet of high dietary energy reached puberty at a younger age than their counterparts fed moderate dietary energy. Supporting their previous data, Hall et al. (1995) reported that beef heifers fed a high-gain diet reached puberty 43 days sooner than those on a moderate gain diet. Increased dietary energy not only decreases age at puberty, but also influences follicular dynamics of young dairy heifers (Chelikani et al., 2003). Gutierrez et al. (1997) reported that the recruitment of small follicles on days 1 and 2 of the estrous cycle increased by 37% when animals were fed twice maintenance ration. They concluded that a short term increase in dietary energy can increase the number of small follicles recruited during the first wave of the estrous cycle in Hereford-Friesian heifers, noting that insulin is closely related to follicle recruitment (Gutierrez et al., 1997). In addition, Bergfeld et al. (1994) reported that composite breed heifers ($\frac{1}{4}$ Angus, $\frac{1}{4}$ Hereford, $\frac{1}{4}$ Red Poll and $\frac{1}{4}$ Pinzgauer) fed larger amounts of dietary energy had larger dominant follicles while heifers fed low dietary energy developed smaller dominant follicles during their estrous cycles. They also noted that first ovulation was, on average, 63 days later in heifers fed low dietary energy.

Timing of weight gains during the prepubertal phase has been studied extensively, due to the expense of feeding a growing heifer from weaning to first estrus. Fleck et al. (1980) reported that heifers with the highest weight gains during the first winter, from weaning to yearling, had the highest final pregnancy rates and noted that rapid growth at this time is beneficial for increased fertility of young heifers. Hall et al. (1997) reported that the percentage of heifers cycling by 12 months of age were greater in animals fed to gain 0.82 kg/day from weaning to the breeding season than animals

fed to first gain 0.41 kg/day for 90 days then 0.82 kg/day. In contrast, Grings et al. (1999) reported that there was little association between altering rates of gains from weaning to the breeding season and reproductive performance of beef heifers.

Numerous studies suggest that delaying the high weight gains until just prior to the breeding season can achieve the same effect as increased weight gains from weaning to the breeding season. If these reports hold true, the knowledge would be valuable to a producer because of the expense of feeding a heifer from weaning to the breeding season. Clanton et al. (1983) suggested that there is flexibility in feeding strategies to achieve timely onset of puberty in beef heifers as long as these animals reach the necessary weight by the beginning of the breeding season. Marston et al. (1995) reported that feeding high concentrate diets for a short period of time prior to the breeding season can reduce age and weight at puberty. Furthermore, Lynch et al. (1997) indicated that delaying the majority of weight gain until late in heifer development can decrease feed costs without detrimental effects on fertility of beef heifers.

Ashworth and Millward (1986) defined compensatory growth in children as the acceleration in growth that occurs when the period of growth restriction ends and favorable conditions are restored. Park et al. (1987) indicated that compensatory growth in dairy heifers can significantly advance tissue development and metabolism including increased rate of gain, increased appetite, while reducing the maintenance requirement of the animal by depressing the basal metabolic rate. Compensatory growth in these dairy heifers also resulted in an advanced endocrine status and improved their overall growth efficiency (Park et al., 1987). Ford and Park (2001) described the effects of compensatory growth on puberty. They concluded that heifers raised on a compensatory nutrition regimen had similar end weights while consuming less feed, which led to improved growth efficiency.

Body composition, or the percentage of body fat to muscle, has also been indicated as a probable influence on age at puberty in beef cattle. In cattle, as in rats, mice, pigs, Rhesus monkeys and humans, a relative percentage of body fat is believed to be required for the onset of puberty (Ahima et al., 1997; Barb et al., 2004; Cheung et al., 1997; Frisch, 1974; Nelsen et al., 1982; Patterson et al., 1992b; Wilson et al., 2003). Yelich et al. (1995) reported that heifers fed to have high weight gains were generally

younger, heavier and fatter than their counterparts that were fed to gain less, while noting that the increased body weight of the high gain heifers was due to fat deposition, since bone and lean weights did not differ among treatments. Hopper et al. (1993) suggested that Angus heifers were physiologically more mature at puberty than Santa Gertrudis heifers, while noting that Angus heifers had more total body fat and thus higher energy reserves. Body fat and leptin will be discussed in a later section due to the importance of the role of leptin as a metabolic and hypothalamic regulator.

Age at Puberty and Nutrition Hormone Relationships

Feed restriction or nutritional deficiency has been found to cause performance and hormonal inadequacies. To discuss the role of nutrition in the onset of puberty, one must bridge the gap between nutrition and endocrine activity. Nutritional anestrus in heifers starts a hormonal chain reaction, reducing blood serum concentrations of Luteinizing Hormone (LH), Insulin-Like Growth Factor-1(IGF-1) and estradiol from the dominant follicle, while ovulation ceases (Armstrong et al., 1992, 2001; Bossis et al., 1999, 2000; Schoppee et al., 1996; Yelich et al., 1996). Resuming alimentation of anestrus animals by increasing nutrition restores hormone levels subsequently resulting in ovulation (Bossis et al., 1999; Wettemann and Bossis, 1999). It has been reported for beef heifers that IGF-1 levels are closely associated with the nutritional status of the animal (Granger et al., 1989; Leon et al., 2004; Spicer et al., 2002; Yelich et al., 1996).

Upon resuming alimentation of nutrition-induced anestrus beef heifers, levels of IGF-1 gradually increases through successive follicular waves. IGF-1 has been associated with increased levels of LH, estradiol and increased size of the dominant follicle, indicating its probable role in the secretion of LH and ovarian function (Bossis et al., 2000). When Growth Hormone Releasing Hormone (GHRH) was inhibited in crossbred heifers, puberty was found to be delayed due to decreased serum IGF-1, which decreases the ability of the ovary to produce preovulatory concentrations of estradiol, thus delaying stimulation of an LH surge (Schoppee et al., 1996). Schoppee et al. (1996) also noted that chronic feed restriction decreased pulse frequency of LH, which may inhibit maturation of follicles to the preovulatory stage. In a similar study,

Yelich et al. (1996) reported that nutrient restriction decreased LH pulse frequency and delayed puberty in beef heifers.

In contrast, Spicer et al. (1991) concluded that reduced ovarian function is not associated with decreased concentrations of plasma IGF-1 and was unlikely the mediator of dietary alterations in ovarian follicular function in Hereford X Friesian crossbred heifers. Day et al. (1986) reported that heifers fed a low plane of nutrition (to gain 0.21 kg/day), had a delayed and less rapid serum LH rise than was commonly found in control animals (fed to gain 0.79 kg/day). They also noted that there was no increase in pulse frequency as found in control animals and LH pulse amplitude also tended to be higher in control animals. Kurz et al. (1990) concluded that restricted energy intake prolonged LH inhibition by estradiol negative feedback in beef heifers that were intact, as well as ovariectomized, and noted that the hypothalamic-pituitary system was restored with increased energy intake. This suggested that energy restriction inhibits the secretion of LH independent of ovarian secretions.

Subsequently, Schillo (1992) reported that inadequate nutrition inhibits the pulsatile secretion of LH by reducing hypothalamic secretion of Luteinizing Hormone Releasing Hormone (LHRH) in cattle and sheep. Bossis et al. (1999) speculated that nutritional alterations influence blood glucose concentrations, which may influence hypothalamic pituitary function in beef heifers. The previous observation is supported by McClure et al. (1978), who reported that injections of 2-deoxyglucose, a known inhibitor of glucose utilization, before and during the estrous cycle in cyclic beef heifers prevented estrus and corpus luteum (CL) formation. Yelich et al. (1996) also concluded that glucose concentrations were positively correlated with LH pulse frequency in prepubertal heifers. Because infusion of glucose in postpartum cows with good body condition did not alter LH secretion, it is concluded that glucose effects on LH secretion are dependent on body condition or degree of fatness and total energy availability in *Bos taurus* crossbred beef cattle (Bossis et al., 1999; McCaughey et al., 1988; Rutter et al., 1989).

Photoperiod, Reproductive Performance and Puberty

Grass et al. (1982) reported that winter conditions during the peripubertal period either delayed or lengthened the onset of puberty. Before cattle were domesticated,

they were once reproductively seasonal animals (Haugan et al., 2005; Jainudeen and Hafez, 2000; Reksen et al., 1999). As many wild type ruminants are, cattle were once long-day breeders. In the wild, cattle would breed to calve during spring months to take advantage of the abundant spring forage (Haugan et al., 2005; Jainudeen and Hafez, 2000; Reksen et al., 1999). Hansen et al. (1983) concluded that even though most cattle are not considered seasonal breeders, age at puberty can be altered by photoperiod, noting that the effect of photoperiod was accompanied by changes in ovarian growth. In the latter experiment, spring-born heifers placed under supplemental lighting after 22 or 24 weeks of age had a reduction in age at pubertal ovulation. In a similar study, Petitclerc et al. (1983) reported that Holstein heifers placed under lights for 16 hours per day for 8 days had increased weight gains, increased feed efficiency and decreased age at puberty regardless of nutrition level.

It has been noted in other experiments that spring-born heifers reach puberty at an earlier age than fall and winter-born heifers (Menge et al., 1960; Roy et al., 1980). Schillo et al. (1983) reported that season of birth and season of puberty procurement interacted to influence age at puberty, indicating that spring then fall environments decreased age at puberty in dairy-beef crossbred heifers. This is consistent with earlier scientific literature indicating that increasing the number of daylight hours decreases age at puberty in beef heifers (Peters and Tucker, 1978). Schillo et al. (1983, 1992) also indicated that beef and dairy crossbred heifers exposure to specific environmental conditions prior to 6 months of life decreased age at puberty, but if exposed to the same set of conditions after 6 months there was a negative effect. It has been reported that photoperiod can alter serum LH and FSH concentrations regardless of plane of nutrition in beef heifers (Critser et al., 1987; Honaramooz et al., 1999).

Most mammals, including cattle, have a diurnal pattern of melatonin release from the pineal gland that increases during the dark hours and decreases during hours of light (Hedlund et al., 1977). In the ewe, this pattern mimics the effects of estradiol negative feedback on LH secretion (Bittman and Karsch, 1984). It is plausible that neural centers involved with LHRH release in cattle could respond to varying levels of melatonin, because of the high binding affinity of melatonin to membranes derived from the medial basal hypothalamus (the location of GnRH neurons) of the bovine brain

(Cardinalli et al., 1979; Kinder et al., 1995; Knobil et al., 1980). Tortonese and Inskeep (1992) also reported that winter-born heifers treated with melatonin reach puberty at an earlier age than their nontreated counterparts of the same age.

Bos indicus breeds of cattle, including crossbreds of *Bos indicus* and *Bos taurus*, are influenced by photoperiod (Mezzadra et al., 1993; Plasse et al., 1968; Stahringer et al., 1990). Stahringer et al. (1990) suggested that cyclic Brahman heifers were found to be seasonal; reporting that winter months had the highest incidence of anestrus. They also noted that cyclic Brahman heifers had transition periods similar to other long-day breeders, such as equids, concluding that these transitional periods seemed to occur before and after months with the highest incidence of anestrus. During these transitional periods, cyclic Brahman heifers had a increased incidence of estrus and ovulation without development of a functional CL (Stahringer et al., 1990).

Endocrine Factors Affecting Puberty

Prepuberty is described as greater than 50 days before the onset of puberty, while peripuberty is described as less than 50 days before the onset of puberty (Kinder et al., 1995). Nakada et al. (2000) first suggested that there are three distinct periods of endocrine maturation that occur during prepubertal and peripubertal heifer development. They also suggested that the first critical period consists of FSH and LH increasing rapidly within a day after birth, noting that this increase was due to the release of inhibition from high levels of estrogens secreted by the placenta while the calf was in utero. It has been reported that increases in FSH, from 12 days of age to 8 months of age, induce follicular waves in prepubertal heifers (Nakada et al., 2000). Increases in FSH in conjunction with the increase in gonadotropins just after birth, which is the first critical endocrine maturation period, play a key role in the initiation of follicular development (Nakada et al., 2000).

Though inhibin, the specific inhibitor of FSH, is known to have an inverse relationship with FSH in postpubertal heifers, Nakada et al. (2000) did not find this relationship in Holstein-Friesian heifers. Also, there has few studies reporting that there is any relation between circulating concentrations of FSH and time of puberty in heifers (Schams et al., 1981; Wolfe et al., 1989). Schams et al. (1981) and Wolfe et al. (1989) noted that the first increase in inhibin was noted between birth and 3 weeks of age.

Then an increase of estradiol, testosterone and inhibin was detected from 4 to 10 weeks of age (Nakada et al., 2000). Ovarian inhibition of LH secretion from the bovine pituitary, via estradiol negative feedback, has been reported to be established by 6 weeks of age (Anderson et al., 1986; Dodson et al., 1989; Moseley et al., 1984; Steffan et al., 1985). It has been reported that the most distinct histological and functional changes in follicular dynamics occurred by 9 weeks of age due to increased numbers of follicles and maximum follicle diameter in beef and dairy heifers (Adams et al., 1994; Desjardins and Hafs, 1969; Dodson et al., 1989; Erickson, 1966). Subsequently, Nakada et al. (2000) concluded that the second key period begins around 4 weeks of age.

In late prepubertal heifers, FSH levels decreased from 15 weeks prior to the pubertal ovulation, while blood serum concentrations of LH and estradiol gradually increased (Gonzalez-Padilla et al., 1975a; Nakada et al., 2000; Schillo et al., 1983). Estradiol negative feedback on LH secretion is most likely one of the primary factors regulating time of onset of puberty in heifers (Day et al., 1984; Foster et al., 1986; Kinder et al., 1987; Roberson et al., 1992). Negative feedback of estradiol on LH secretion via a decrease in GnRH pulses gradually decreases until the onset of puberty in cattle. This is thought to be the effect of a decreased number of estradiol-17 β receptors or estradiol binding sites in the hypothalamus (Day et al., 1987; Kinder et al., 1987). Opioid peptides have been implicated in prepubertal LH suppression by increasing the sensitivity of estradiol at the medial basal hypothalamus by increasing the number of estradiol-17 β receptors in the hypothalamus in cattle and sheep (Armstrong and Johnson, 1989; Byerley et al. 1992; Evans et al., 1992; Kinder et al., 1987; Wolfe et al., 1991, 1992). These findings are consistent with others stating that LH concentrations gradually rise as heifers approach puberty due to a decrease in estradiol negative feedback (Day et al., 1984; Dyer et al., 1990; Gonzalez-Padilla et al., 1975a; Rodrigues et al., 2002; Schillo et al., 1982). In beef heifers, ovarian inhibition of LH pulses by estradiol negative feedback is most noticeable after bilateral ovariectomy and exogenous estradiol administration (D'Occhio et al., 1988; Day et al., 1984; Dyer et al., 1990; Moseley et al., 1984; Roberson et al., 1992; Rodrigues et al., 2002; Schillo et al., 1982; Steffan et al., 1985; Stumpf et al., 1992).

In ovariectomized prepubertal beef heifers, LH pulse frequency reaches 1 pulse per hour by 49 days postovariectomy, which is a LH pulse characteristic of beef heifers approaching puberty (Moseley et al., 1984). During prepuberty (>50 days prior to onset of puberty), LH pulse frequency was found to be 1 to 4 pulses per 24 hours, while during peripuberty (<50 days prior to the onset of puberty), LH pulse frequency gradually increases to 1 pulse per hour or 24 pulses in 24 hours at just prior to the onset of puberty in crossbred beef heifers (Day et al., 1987). These findings are consistent with others stating that there is a transient rise in circulating LH just prior to puberty in cattle (D'Occhio et al., 1988; Hansen et al., 1983; Page et al., 1987).

In contrast, Gonzalez-Padilla et al. (1975a) suggested that the levels of LH do not gradually increase as puberty approaches but actually decrease and fluctuate within a smaller range, especially during the 6 days prior to the pubertal LH peak. Subsequently, Nakada et al. (2000) concluded that the period just prior to puberty is the third key period in the regulation of endocrine maturation and physiological changes before the onset of puberty.

Though the pubertal LH peak can be induced in a prepubertal heifer, there seems to be one or more components of the endocrine system that are not capable of functioning in an adult fashion until the onset of puberty (Dodson et al., 1988; Grasselli et al., 1993). Grasselli et al. (1993) noted that a pubertal LH peak could be induced by GnRH infusion in prepubertal Holstein heifers, but ovulation with a subsequent post-treatment rise in progesterone did not occur.

Progesterone has been associated with the onset of puberty in beef heifers and has been indicated in playing a key role in the physiological changes in LH release from immature status to that characteristic of a mature animal, by reducing the effects of estradiol negative feedback on the hypothalamus. There are two distinct elevations in progesterone prior to the pubertal LH peak (Gonzalez-Padilla et al., 1975a). Gonzalez-Padilla et al. (1975a) also noted a large degree of association between LH and progesterone despite its lack of significant correlation. They found a short elevation of progesterone followed by a small priming LH peak after the first progesterone elevation had reached baseline levels. These elevations in progesterone and LH trigger a subsequent greater rise in progesterone, presumably of ovarian origin, followed by the

pubertal LH peak, which resulted in development of a palpable CL. Gonzalez-Padilla et al. (1975a) also noted that the LH levels between the priming LH peak and the pubertal LH peak, coincident with the second progesterone elevation, seemed to be a transition period between pubertal and postpubertal LH baseline levels. These two peaks of progesterone prior to the onset of puberty have also been reported in Yak heifers (Yu and Li, 2001).

There is some conflicting literature on the source of the first elevation of progesterone. Gonzalez-Padilla et al. (1975a) concluded that it was possible that the first progesterone elevation was at least partially produced by the adrenal cortex. This is probable because it has been reported that adrenal venous blood in cattle contains a 10-fold increase in progesterone when compared with arterial blood (Balfour et al., 1957). Schams et al. (1981) also stated that blood progesterone levels before 9 months of age seem to be of adrenal origin. In contrast, Berardinelli et al. (1979) concluded that the first elevation in progesterone prior to puberty in beef heifers was produced by short-lived luteal tissue embedded within the ovary, not palpable and only visible by microscopic dissection, while suggesting that the tissues were luteinized follicles. Though some of these structures were solid and compact, not characteristic of a luteinized follicle, which has an antral cavity lined with luteal tissue, it is probable that these structures were derived from follicles that had not yet developed an antrum (Berardinelli et al., 1979). This phenomenon has also been reported for ewes (Berardinelli et al., 1980).

There have been other mechanisms recently implicated in physiological and endocrine maturation of the prepubertal and peripubertal bovine female. This review refers to these mechanisms as maturational blocks or mechanisms that inhibit the release of LH that is characteristic of a mature bovine female. One of the most recently discovered blocks on LH pertains to leptin, neuropeptide-Y (NPY) and opioid effects at the hypothalamic and pituitary levels. The most marked endocrine change in heifers at the onset of puberty is the change in the effect of estradiol on LH secretion. During prepuberty, estradiol inhibits proper LH pulsatile secretion and after the pubertal LH peak, enhances LH secretion. Day et al. (1986) indicated that the well documented decline in estradiol negative feedback during prepuberty and peripuberty is followed by

a period of positive feedback on LH secretion after the pubertal LH peak. As estradiol negative feedback declines, the LH pulse frequency increases allowing follicles to develop to larger more advanced stages. The increase in advanced stage follicles produce high levels of estrogen, which stimulates marked uterine growth and development (Kinder et al., 1987). Kinder et al. (1995) also indicated that increased estradiol from larger follicles that develop during peripuberty combine with transient increases in progesterone to induce significant changes in uterine morphology during the last stages of sexual maturity. At this stage LH concentrations are high enough, a frequency of 1 pulse per hour, to drive follicular growth to the preovulatory stage, also noting that the high levels of estradiol produced from these follicles are enough to elicit a preovulatory surge of gonadotropins resulting in ovulation or luteinization of an antral follicle.

It has been shown that endogenous opioids may play a role in the inhibition of LH secretion during sexual maturation. Wolfe et al. (1991) reported that opioid neuropeptides and estradiol act together to regulate LH and FSH secretion during sexual maturation in heifers, but noted that as heifers move from prepuberty to peripuberty, the effects decline. They also noted that opioid neuropeptides and estradiol interact to inhibit gonadotropin secretion during prepuberty and the decline in estradiol negative feedback during sexual maturation is associated with or linked to the decline in opioid inhibition of gonadotropin secretion (Wolfe et al., 1991, 1992).

Wolfe et al. (1992) advanced this physiological model, indicating that opioid inhibition of gonadotropin secretion declined during peripuberty but remained intact in ovariectomized heifers treated with exogenous estradiol after their intact counterparts of the same age had already attained puberty. They also noted that opioid inhibition is important in regulating gonadotropin secretion in peripubertal heifers and continue to be involved in the regulation of circulating LH concentrations after the onset of puberty (Wolfe et al., 1992). Armstrong and Johnson (1989) suggested that administration of an exogenous opioid receptor agonist inhibited episodic release of LH in heifers. To support the previous study, Byerley et al. (1992) concluded that opioid inhibition of LH secretion is functional in heifers from the prepubertal period to the luteal phase of the pubertal estrous cycle, reporting that serum LH concentrations increase within 30

minutes after naloxone (opioid antagonist) administration suggesting that opioids are related to the events leading to puberty in heifers. Other studies also reported an increase of serum concentrations of LH after naloxone treatment (Gregg et al., 1986; Whisnant et al., 1986). The results of Byerley et al. (1992) support the hypothesis that the changes in estradiol receptor numbers or type may be under opioid control and also support a role of opioids in the tonic suppression of LH release. In contrast, Evans et al. (1992) reported that opioid control of gonadotropin secretion occurs in 4 week old calves, but after that period the action is reduced or absent.

Scientists have known of a link between nutritional status and the endocrine system for a number of years. The scientific literature indicates that nutrition is the key determinant of reproductive potential in cattle as well as other animals including humans. This relationship has been implicated in the timing of onset of puberty and maintenance of normal reproduction in many species. Leptin, a 16 kDa peptide produced in white adipocytes, was discovered in mice as the product of the obesity gene (*ob*) and is now well understood. Lack of leptin is associated with increased feed intake, reduced energy expenditure, obesity and infertility in mice (Apter, 1997; Halaas et al., 1995; Weigle et al., 1995). Leptin has been found to have the ability to trigger the onset of puberty in rodents and to make mutant *ob/ob* mice (genetically obese mice that lack leptin production) fertile, who otherwise would be sterile (Barash et al., 1996; Chehab et al., 1997). Cheung et al. (1997) suggested that leptin is a metabolic gate for the onset of puberty in the female rat, stating that leptin is not the primary signal that initiates the onset of puberty, but instead, acts in a permissive fashion to allow pubertal maturation to proceed.

In humans, leptin may act as the critical link between adipose tissue and the reproductive system, indicating whether adequate energy reserves are present for normal reproductive function (Moschos et al., 2002). In livestock species, poor nutrition is associated with infertility and delayed puberty. Feed restricted prepubertal animals will not attain puberty until their plane of nutrition is increased. Additionally, cyclic cows and ewes will go into anestrus when faced with extreme undernutrition. Energy restriction delays the onset of puberty primarily by preventing the development of high frequency LH pulses due to lack of proper GnRH stimulation, increased sensitivity to

estradiol negative feedback and increased tonic levels of opiates at the medial basal hypothalamus (Kinder et al., 1987).

Prasad et al. (1993) concluded that decreased caloric intake decreases LH pulsatile release and prevents onset of puberty in prepubertal lambs by decreasing the amplitude of LHRH output from the hypothalamus. It has been reported that body weight, time of onset of puberty, and circulating leptin are highly correlated, noting that serum concentrations of leptin increases linearly from 16 weeks of age until pubertal LH peak in yearling beef heifers reaching sexual maturation from early spring to mid-summer (Garcia et al., 2002). Leptin mRNA also increased in adipose tissue as beef and dairy heifers approached puberty, while increasing leptin levels during sexual development parallel increases in body weight (Diaz-Torga et al., 2001; Garcia et al., 2002). Amstalden et al. (2000) concluded that fasting decreases leptin mRNA, as well as, circulating leptin coincident with decreases of IGF-1, insulin and LH in prepubertal beef heifers.

The hypothalamus was among the first tissues found to contain the long form of the leptin receptor (*Ob-R*), the only active signaling form, mRNA (Spicer, 2001; Tartaglia et al., 1995). It has been reported that leptin receptors have been localized with NPY, a potent stimulator of food intake and inhibitor of LH in cattle and sheep, neurons in rodents and sheep, indicating that there may be a direct effect of leptin on NPY (Garcia et al., 2002; Keisler et al., 1999; Mercer et al., 1996). This pathway has been confirmed by the decreased intake of ewes (well fed and feed restricted) when given intracerebroventricular infusions of leptin (Henry et al., 1998; Morrison et al., 1998). It has been reported that leptin induced drastic reductions in food intake and body weight in mutant mice lacking the NPY-Y1 receptor, as well as wild type mice, noting that there was a marked decrease in age at puberty in the mutant mice treated with leptin (Gonzales et al., 2004; Pralong et al., 2002).

Furthermore, administration of leptin also prevented the decrease in LH concentration in feed restricted wethers (Foster and Nagatani, 1999). Supporting the previous experiment in sheep, Amstalden et al. (2002) reported that central administration of leptin to fasted cows increased plasma LH, noting that the increase was due to an increase in LH pulse amplitude. Carro et al. (1997) also indicated that

ovariectomized rats administered leptin antiserum were found to have a decrease in LH secretion. Subsequently, Maciel et al. (2004a) reported that administration of exogenous leptin could modify GnRH-mediated release of LH in fasted prepubertal beef heifers, preventing the reduction in LH pulse frequency normally found in fasted animals. Also, Maciel et al. (2004b) reported that chronic administration of recombinant ovine leptin failed to either induce puberty or alter endocrine profiles in prepubertal beef heifers on a normal plane of nutrition. These data are consistent with a previous report in rats, which suggested that leptin acts only in a permissive fashion to allow pubertal maturation to proceed (Cheung et al., 1997).

Though there are many other potential mediators of leptin in the mediobasal hypothalamus, there are none more probable than NPY. The synthesis of NPY in the arcuate nucleus is inhibited by leptin, which leptin is known to decrease food intake before any change in body weight and increase fertility in sterile *ob/ob* mice (Cusin et al., 1996; Erickson et al., 1996; Houseknecht and Portocarrero, 1998; Schwartz et al., 1996; Stephens et al., 1995). It is plausible that leptin is the major hormone signaling energy or nutritional status to the neurohormonal reproductive axis in cattle.

Results reported by Amstalden et al. (2000) indicate that Growth Hormone (GH) could indirectly stimulate leptin production. Houseknecht et al. (2000) found that GH increased leptin concentrations in adipose tissue, while also increasing IGF-1 mRNA concentrations, noting that GH induced these changes before measurable GH effects on adiposity.

Recently, body condition has been associated with circulating leptin levels. Leon et al. (2004) concluded that leptin was a direct indicator of nutritional status while serving as a dynamic indicator of body condition in heifers. Leon et al. (2004) also stated that circulating leptin levels paralleled body condition score (BCS) in heifers and was positively correlated with IGF-1 and insulin during weight gain. It seems that leptin is a pivotal hormone that acts to orchestrate a complex array of signals that regulate food intake, energy expenditure, nutrition partitioning, as well as, reproductive function in cattle.

It is probable that leptin influences reproduction in cattle, not only at the hypothalamic level, but also directly at the adrenal and ovarian levels. Cortisol is a

known inhibitor of pulsatile LH release via its block on LHRH release (Armstrong and Johnson, 1989; Hoffman et al., 1996; Kujjo et al., 1995; Li and Wagner, 1983a; Li and Wagner, 1983b; Matteri and Moberg, 1982; Stoebel and Moberg, 1982). This is noted in postpartum cows that have been suckled (Dunlap et al., 1981a, 1981b; Hoffman et al., 1996; Padmanabhan et al., 1983). Hoffman et al. (1996) concluded that calf presence associated with increased cortisol levels is one factor that increases time to first postpartum ovulation in beef cows. Cortisol has also been reported to suppress progesterone secretion from luteal tissue via its inhibition of episodic LH release (Li and Wagner, 1983a). Li and Wagner (1983b) also stated that cortisol inhibits LH secretion from pituitary cells even when challenged with GnRH. Administration of exogenous cortisol can delay the preovulatory LH surge, while also inhibiting estrous behavior (Stoebel and Moberg, 1982). Dunlap et al. (1981a, 1981b) reported that postpartum cows administered exogenous ACTH had a subsequent rise in serum cortisol and inhibition of LH release. Correspondingly, Ribadu et al. (2000) successfully induced follicular cysts in four of five heifers injected with exogenous Adrenal Corticotropin Hormone (ACTH), indicating that cortisol levels were increased during ACTH treatment. It has also been reported that dexamethasone, a synthetic glucocorticoid, acts via the same mechanism as cortisol, reducing pulsatile LH secretion (Vighio and Liptrap, 1990).

It has been reported that leptin can inhibit cortisol production directly at the adrenal level (Bornstein et al., 1997). Bornstein et al. (1997) concluded that leptin inhibited basal and ACTH stimulated cortisol release, clearly demonstrating that leptin inhibits cortisol production directly in adrenocortical cells. Opioid peptides have also been indicated in the stimulation of cortisol production in the adrenal gland (Armstrong and Johnson, 1989). Therefore, leptin, via a second pathway, may also inhibit cortisol production by inhibiting opioid influence on adrenal steroidogenesis.

Leptin has also been indicated to act directly on the ovary to decrease estradiol production in follicular granulosa cells. Spicer and Francisco (1997) concluded that physiological levels of leptin can directly inhibit insulin-induced steroidogenesis of granulosa cells without affecting their mitotic division, while suggesting that granulosa cells may have high-affinity receptors for leptin. Spicer and Francisco (1998) took this information a step further, indicating that leptin can also inhibit insulin-induced thecal

cell steroidogenesis. They stated that the inhibitory effect of leptin was not mediated through inhibition of insulin binding, but rather leptin binding to its own receptor.

Sexual Maturation Anomalies

Anomalies that occur during sexual maturation in heifers are common events. The two most common are nonpubertal estrus and precocious puberty. Nonpubertal estrus is a phenomenon described as behavioral estrus in prepubertal heifers that is followed neither by ovulation or formation of a CL. Certain physiological changes involved in sexual maturation may trigger behavioral estrus before all maturational events leading to puberty are complete (Nelsen et al., 1985). Nelsen et al. (1985) stated that nonpubertal estrus may be influenced by age, photoperiod, genotype or breed type. The prevalence of nonpubertal estrus has been reported to occur in 22 to 62% of beef heifers (Byerley et al., 1987a; Nelsen et al., 1985; Rutter and Randel, 1986). Though nonpubertal estrus has not been reported to affect fertility of heifers, it can be a problem when using behavioral estrus as the sole criteria for assigning date of puberty onset for replacement heifer selection or research studies (Nelsen et al., 1985; Rutter and Randel, 1986).

Another anomaly, precocious puberty, is the attainment of puberty early in the prepubertal period of the female. It has been reported that precocious puberty may be caused from the neonatal period, during which estradiol negative feedback on the hypothalamus inhibits pulsatile LH, being extended until later in life (Wehrman et al., 1996). If bulls are present during this time, when the heifer has a precocious estrous cycle, it could result in the heifer becoming pregnant at an earlier than normal age. This could pose a problem because heifers will calve at an inadequate body size, increasing the chance for dystocia. Furthermore, these precocious puberty heifers normally conceive late in the breeding season and calve after the normal breeding season as yearlings. As many as 25% of heifers may exhibit transient cyclic luteal function prior to 300 days of age, indicating that precocious puberty in beef heifers is not an uncommon event (Wehrman et al., 1996).

Reproductive Maturation after the Pubertal LH Peak

Swanson et al. (1972) suggested that reproductive maturation continues for numerous estrous cycles after the onset of puberty, before endocrine stability is

achieved, noting that serum LH concentration during the luteal phase of the estrous cycle significantly declined from first to the seventh cycles. Morrow et al. (1970) also stated that anovulatory cycles were greater in number among first and second estrous cycles than among subsequent cycles. Byerley et al. (1987a) reported that fertility of the third estrous cycle is higher than the pubertal estrous cycle in beef heifers, while suggesting that the higher fertility of the third estrous cycle was related to maturational changes that occur following the pubertal estrous cycle in beef heifers. In the latter experiment, pregnancy rates were significantly higher in beef heifers mated during their third estrous cycle than ones mated on their pubertal estrous cycle. Byerley et al. (1987a) concluded that the difference in pregnancy rates was due to early or late embryonic mortality, because there was no difference in ovulation rate with subsequent rise in progesterone from day 6 to day 12 of the estrous cycle.

In a second experiment, Byerley et al. (1987b) analyzed progesterone concentrations from first estrous cycle to third estrous cycle in beef heifers and they concluded that luteal function differed between pregnant and nonpregnant heifers mated at pubertal or third estrus. It has been reported that progesterone concentrations are higher in pregnant heifers than in nonpregnant heifers 12 days postbreeding, while suggesting that a change in the progesterone to estrogen ratio between the first and third estrous cycles contributed to the high embryonic mortality (Breuel et al., 1989; Byerley et al., 1987a, 1987b; Chagas e Silva and Lopes da Costa, 2005; Henricks et al., 1971; Jaster et al., 1982). Byerley et al. (1987b) also reported that the high concentration of progesterone reported for nonpregnant heifers during their pubertal estrus was produced by short-lived luteal tissue, while inadequate uterine development and ovarian function were also probable contributors to the high embryonic mortality found in nonpregnant beef heifers (Byerley et al., 1987b). More insight into luteal function during the first three estrous cycles was reported by Del Vecchio et al. (1992b).

Del Vecchio et al. (1992b) accounted for a high frequency of abnormal cycle lengths in dairy heifers during the first two estrous cycles when compared with heifers in their third estrous cycle, which had 0% abnormalities. It was also concluded that peak hormone timing and anomalies in uterine and ovarian endocrine activity during the

pubertal period in heifers played a major role in the endocrine dysfunction found in dairy heifers during their first and second estrous cycles.

More recent studies have investigated the physiological and endocrinological relationship between the uterus and ovaries in the prepubertal heifer. Treatment of mature cows with oxytocin has been shown to elicit a rapid release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus (Del Vecchio et al., 1990; Silvia and Taylor, 1989). It has been suggested that the prepubertal bovine uterus may respond to oxytocin between 6 and 9 months of age and by 10 to 11 months, it is possible to obtain 100% response to oxytocin (Del Vecchio et al., 1991). In a second study, there was no increase in prostaglandin F metabolite (PGFM) (which is measured to indicate $PGF_{2\alpha}$ levels produced by the uterus) when beef heifers were treated with oxytocin, but there was a marked increase in concentration of PGFM when heifers were treated with progesterone for 9 days prior to an oxytocin challenge (Del Vecchio et al., 1992a). It was suggested that mimicking the prepubertal rise in progesterone would elicit an increase of PGFM, while enhancing the sensitivity of the uterus to oxytocin. Consistent with information found in ovariectomized ewes, the data suggests that an increase in progesterone (for periods of 7 days or longer) may increase the number of oxytocin receptors in the uterus (Del Vecchio et al., 1992a; Vallet et al., 1990).

Further investigation indicated that oxytocin mediates the prostaglandin release from the uterine endometrium via induction of Prostaglandin Hormone Synthase-2 (PGHS-2 gene also known as Cyclooxygenase II or COX-2) transcription. The oxytocin receptor gene is present throughout puberty as is the PGHS-1 (COX-1) gene. Low estrogen levels present in prepubertal heifers just prior to the onset of puberty may enhance oxytocin receptor population indirectly by promoting uterine and cervical maturation. It is now thought that oxytocin does not elicit a prostaglandin response from the uterus because the uterus does not express COX-2 transcription before puberty in beef heifers (Fuchs et al., 1998). Therefore, it is believed that the progesterone increase, 1 to 3 weeks prior to puberty, may prepare the uterus to perform the endocrine function of producing $PGF_{2\alpha}$ that may be necessary for normal ovarian cyclicity and function (Del Vecchio et al., 1992).

Induction of Puberty

Inducing puberty could be a profitable tool for a cattle producer. Age at puberty is an important production characteristic when heifers are bred to calve at 2 years of age within restricted breeding systems. Heifers that calve at 2 years of age produce one more calf per lifetime than their counterparts that calve at 3 years of age and heifers that are mated and conceive early within their first breeding season will continue to calve earlier and wean heavier calves throughout their lifetime than those who conceive later (Lesmeister et al., 1973). If a beef producer can control the onset of puberty, a management decision to breed heifers during their first breeding season will ultimately result in profit. Even though age at puberty can be modified by genetic variables and feeding strategies, a large percentage of yearling heifers fail to reach puberty by the start of the first herd breeding season.

Exogenous progestogens have been used in numerous reports to induce puberty in prepubertal heifers (Ahmad et al., 1996; Anderson et al., 1996; Ghallab et al., 1984; Gonzalez-Padilla et al., 1975c; Grings et al., 1998; Imwalle et al., 1998; Jaeger et al., 1992; Sanchez et al., 1995; Sheffield and Ellicot, 1982; Whisnant and Burns, 2002). Gonzalez-Padilla et al. (1975c) were among the first groups to note the efficacy of an exogenous progestogen to induce the onset of puberty in beef heifers. They noted that if combined with estrogen, the use of progestogens could mimic changes that occur in blood hormone concentrations at the onset of puberty. Though the treatment proved effective in beef heifers approaching puberty, Gonzalez-Padilla et al. (1975c) also concluded that their treatment may prove ineffective in heifers that are far from the date of expected onset of puberty. Correspondingly, Sheffield and Ellicot (1982) indicated that low levels of exogenous progesterone will induce behavioral estrus in prepubertal beef heifers following its withdrawal. Ghallab et al. (1984) used repetitive norgestomate (a strong synthetic progestogen) to induce puberty in beef heifers, noting that the treatments increased pregnancy rates in yearling beef heifers after a 24 day breeding season by 27.6%.

The exogenous progestogen, melengestrol acetate (MGA), has also been used in conjunction with PGF_{2α} to effectively induce puberty in beef heifers (Jaeger et al., 1992). They noted that MGA+PGF_{2α} treatment not only induced puberty in heifers, but

effectively synchronized estrous cycles and enabled a higher percentage (49% treated vs. 14% untreated) of animals to become pregnant early in the breeding season.

It has been reported that norgestomate (4 norgestomate implants equivalent to 24 mg of norgestomate) can mimic the mature mid luteal phase concentration of progesterone with respect to modulation of LH pulses, rate of ovarian follicular growth, secretion of estradiol, size of the preovulatory follicle and time to the preovulatory LH surge in beef heifers (Sanchez et al., 1995). Anderson et al. (1996) reported that 86% of prepubertal crossbred beef heifers (320 and 322 days of age) in their experiment achieved puberty after administration of one 6 mg norgestomate implant for 10 days. They also noted dramatic uterine growth, increased LH secretion and the advancement of pubertal ovulation accompanied withdrawal of the implant, which has also been reported for a 14 day administration of MGA (Anderson et al., 1996; Hill et al., 1971; Smith and Day, 1990). Anderson et al. (1996) concluded that the beef heifers that did not attain puberty after implant removal may not have reached the critical time point of the initiation of the decline in estradiol negative feedback that marks the transition from prepuberty to peripuberty, suggesting that the efficacy of exogenous progestogens is dependent upon the timing of administration in relation to the initiation of the decline in estradiol negative feedback on the hypothalamus. A similar percentage of beef heifers attaining puberty due to norgestomate administration have also been reported (Grings et al., 1998).

MGA, fed for 14 days, is known to enhance the onset of puberty by stimulating pulsatile LH secretion, via a decrease in estradiol negative feedback on the hypothalamus, which is needed for follicle advancement to the preovulatory phase in beef heifers (Imwalle et al., 1998). A contrasting report concluded that first service pregnancy rates were reduced after a 7 day MGA treatment followed by a PGF_{2α} on day 7, but suggested that their reports could have been influenced by prebreeding nutritional status (Patterson et al., 1989). Further research indicates that the results from Patterson et al. (1989) may not be due to just prebreeding nutritional status. Heifers treated with MGA often develop persistent dominant follicles that do not ovulate during treatment (Anderson and Day, 1994; Custer et al., 1994; Imwalle et al., 2002; Yelich et al., 1997). After MGA withdrawal and preovulatory LH surge, the ovulated ovum may

be nonviable. MGA apparently does not inhibit pulsatile release of LH and the persistence of high frequency LH pulses combined with the absence of ovulation during MGA treatment suggests that MGA only blocks the preovulatory surge of LH, enabling the ovum to age to a nonviable state (Imwalle et al., 2002; Kojima et al., 1995). This finding is of major importance, because the results of Imwalle et al. (2002) support the hypothesis that there are different neuropathways involved with basal and surge models of LH release in cattle.

Ionophore administration has also been indicated in advancing the onset of puberty in heifers. Many studies have associated the alteration of ruminal fermentation, when fed sodium monensin, with decreased age at puberty in heifers. The alteration in ruminal fermentation refers to the increased production of ruminal propionate over ruminal acetate. The volatile fatty acid propionate is the single most important substrate for glucose synthesis in ruminants. McCartor et al. (1979) concluded that treatments which altered ruminal fermentation towards increased levels of ruminal propionate and decreased levels of acetate could significantly increase the percentage of beef heifers that would conceive by 15 months of age, enabling them to calve by 24 months of age. Beef and dairy heifers fed sodium monensin, at 200 mg/day, can conceive 21 to 38 days sooner than their untreated counterparts (Baile et al., 1982; Lalman et al., 1993; Meinert et al., 1992). The reduction in age at puberty in heifers fed monensin, independent of growth rate, has been reported in numerous studies (Baile et al., 1982; Lalman et al., 1993; Meinert et al., 1992; Moseley et al., 1982; Purvis and Whittier, 1996).

There is evidence suggesting that monensin may affect gonadotropin production by a direct or indirect influence on the ovary and hypothalamic-pituitary axis. Bushmich et al. (1980) reported that beef heifers fed sodium monensin developed a significant increase in ovarian response to gonadotropins, including greater ovarian weight, greater total luteal weight, more follicles, greater follicular fluid and greater follicular stromal weight. Randel and Rhodes (1980) reported an increase in the amount of LH released after GnRH challenge in prepubertal beef heifers fed monensin, noting that dietary monensin increases the capability of the pituitary to release LH. Randel et al. (1982) took this finding a step further, stating that monensin can alter the estradiol-induced LH

surge in prepubertal crossbred beef heifers, reporting an increase in peak LH concentration, as well as, an increase in the duration of the LH surge. It has also been reported that monensin can increase the number of ova recovered per cow, increase the number of CL developed and increase total luteal weight in crossbred beef cows during a superovulation protocol (Ortuno and Carson, 1985).

Armstrong and Spears (1988) were the first to provide evidence of the effect of monensin on ruminant metabolism, independent of ruminal volatile fatty acid alterations, through intravenous administration of monensin to beef heifers. In contrast to other reports demonstrating positive effects of monensin on the endocrine function of prepubertal heifers, Armstrong and Spears (1988) reported that intravenous administration of monensin suppressed LH release but they provided direct evidence that monensin can affect serum concentrations of insulin, fatty acids, potassium, magnesium, phosphorus and LH that is independent of ruminal alterations of volatile fatty acids.

In summary, to obtain beef heifers that conceive early within their first breeding season, they must achieve puberty and have two or three estrous cycles prior to the breeding season. The heifers must reach a target weight, 65% of the mature weight, one to two months prior to the breeding season. The animals must be in good body condition, but not overly fat prior to the breeding season. Also, a beef producer can utilize certain management tools to increase the percentage of heifers reaching puberty one to two months prior to the breeding season such as genetic selection, crossbreeding, season of birth, exogenous progestogens and feeding ionophores.

CHAPTER III

THE ADDITIVE EFFECT OF MELENGESTROL ACETATE (MGA) PRIMING AND SODIUM MONENSIN ON REPRODUCTIVE PERFORMANCE IN BEEF HEIFERS

Introduction

Breeding beef heifers to produce calves at 2 years of age can be a profitable management decision (Lesmeister et al., 1973). Until now, methods to obtain this have proved to be inefficient. When at the end of a 60-day breeding season, current practices normally result in too few pregnant heifers. The current practices, more often than not, produce either nonpregnant animals or animals that conceive late in the breeding season.

A multitude of methods have been attempted to increase the efficiency of yearling heifers conceiving by 15 months of age. Most attempts made have been directed at nutrition, environment and other management practices. In addition, chemical agents and hormone therapies have been instituted at various stages of development trying to increase the percentages of yearling heifers having normal estrous cycles prior to the breeding season. To the author's knowledge, there has not been a published experiment in which developing heifers have been subjected to an oral progestogen for multiple cycles, attempting to mimic the normal estrous cycle of a mature cow. It is hypothesized that taking this approach will aid in the maturation of the endocrine system of developing beef heifers.

The ionophore, monensin, has also been utilized in the past to increase the number of yearling heifers cycling normally prior to the breeding season, but most experiments have been directed towards feed efficiency and increased planes of nutrition (Baile et al., 1982; Lalman et al., 1993; McCartor et al., 1979; Meinert et al., 1992; Moseley et al., 1982; Purvis and Whittier, 1996). Few experiments have investigated a synergistic effect between monensin and exogenous progestogens and even fewer have directed their attention to the extraruminal effects of monensin (Armstrong and Spears, 1988; Bushmich et al., 1980; Randel and Rhodes, 1980; Randel et al., 1982). It is hypothesized that introducing an exogenous progestogen treatment to an existing monensin treatment will have a synergistic effect on endocrine maturation of yearling beef heifers.

The goal of this experiment was to enhance the fertility of replacement heifers at the time of breeding (prior to 15 months of age) and to obtain a high percentage of animals pregnant that had conceived early in the breeding season. The overall purposes of this study were (1) to examine the effects of repeated progestogen priming on the attainment of puberty in prepubertal beef heifers, (2) to evaluate the potential additive effect of melengestrol acetate (MGA) plus sodium monensin on attainment of puberty in beef heifers, (3) to examine subsequent reproductive performance, including the response to synchronization of estrus, first service pregnancy rate and overall pregnancy rates, and (4) to examine the effects of repeated progestogen treatments on follicular dynamics of prepubertal beef heifers.

Materials and Methods

The objective of this study was to examine the effects of repeated progestogen priming on the attainment of puberty in prepubertal heifers. Other objectives include evaluating the potential additive effect of progestogen priming plus sodium monensin on attainment of puberty in heifers, and to examine their subsequent reproductive performance. This includes the response to synchronization of the estrous cycle, first service pregnancy rate and overall pregnancy rates. Also, to examine the effects of repeated progestogen priming on follicular dynamics of prepubertal beef heifers. One experiment, with two replicates, was conducted at Louisiana State University Idlewild Experiment Station. Replicate 1 was conducted from September 9, 2002 to July 22, 2003. Replicate 2 was conducted from September 10, 2003 to July 22, 2004. Each replicate started at weaning and ended at pregnancy determination.

Experimental Animals

Eighty-five crossbred beef heifers ($\frac{1}{4}$ Brahman, $\frac{1}{4}$ Hereford, $\frac{1}{2}$ Angus) (Figure 3.1) were weaned and preconditioned for 84 days. Preconditioning included vaccination with Pyramid[®] 4 (IBR, BVD, PI-3, BRSV, modified live respiratory vaccine; Fort Dodge Animal Health, Fort Dodge, Iowa) and Fortress[®] 7 (*Clostridium chauvoei-septicum-novy-sordellii-perfringens* types C&D bacterin/toxoid vaccine; Pfizer Animal Health, Exton, PA) and the feeding of a 90% cracked corn and 10% soybean meal ration (~12% crude protein) at 2.3 kg per head per day. Upon completion of the preconditioning period, heifers were placed into one of four groups that were stratified by the adjusted



Figure 3.1. A photo of a group of the crossbred beef heifers used in this experiment at Louisiana State University, Idlewild Experiment Station. This figure shows the homogeneity of the experimental animals.

weaning weights. The four groups were randomly assigned one of four treatments (Treatment A = Control, Treatment B = MGA, Treatment C = Rumensin, Treatment D = MGA+Rumensin). Each group was allotted to similar sized pastures (approximately 1 hectare) containing a mixture of Bermudagrass and Bahia grass during the spring, summer and fall months from April to October.

The pastures contained annual ryegrass during the winter months from November to March. The animals had access to ad libitum water throughout the experiment and were supplemented with good quality Bermudagrass hay during periods of low rainfall. Also, all animals had access to ad libitum loose macrominerals (calcium, magnesium, phosphorus, potassium, sodium, chloride and sulfur).

All heifers were the offspring from the same herd of crossbred beef cows and sired by the same Angus bulls for Replicate 1 and Replicate 2. Average birth weight of the experimental animals for both Replicate 1 and Replicate 2 was 36.2 kg. Hip heights were recorded and frame scores were assigned to heifers when the average age of the animals was ~365 days for Replicate 1 and ~205 days for Replicate 2 (shown in Figures 3.2 and 3.3). The frame score formula was adopted from Dhuyvetter (1995) and consisted of: $\text{Frame Score} = -11.7086 + 0.4723 (\text{Hip Height}) - 0.0239 (\text{Days of Age}) + 0.0000146 (\text{Days of Age})^2 + 0.0000759 (\text{Hip Height}) (\text{Days of Age})$. This formula adjusts for age of the animal to remove that variable, allowing the hip height to be taken at 205 days or 365 days to achieve an accurate frame score.

Body weight data and body condition scores were collected on 28-day intervals of the study along with jugular vein blood samples. Body condition scores were given based on a scale of 1 to 9, with a score of 1 being extremely emaciated and a score of 9 being morbidly obese (Richards et al., 1989). Some minor adjustments between Replicate 1 and Replicate 2 were needed due to animal acquisition constraints but these adjustments did not affect the ability of this project to be conducted as replicates. The adjustments were crossbred beef heifers (n = 45) were weaned on September 9, 2002 for Replicate 1 and crossbred beef heifers (n = 40) were weaned on September 10, 2003 for Replicate 2.

Experimental Procedure

The heifers in the Control group were fed a 12% crude protein supplement consisting of 90% cracked corn and 10% soybean meal at a rate of 2.3 kg per head per



Figure 3.2. Photo of the cattle hip height measuring device located inside the cattle handling chute at Louisiana State University, Idlewild Experiment Station. November, 2002.

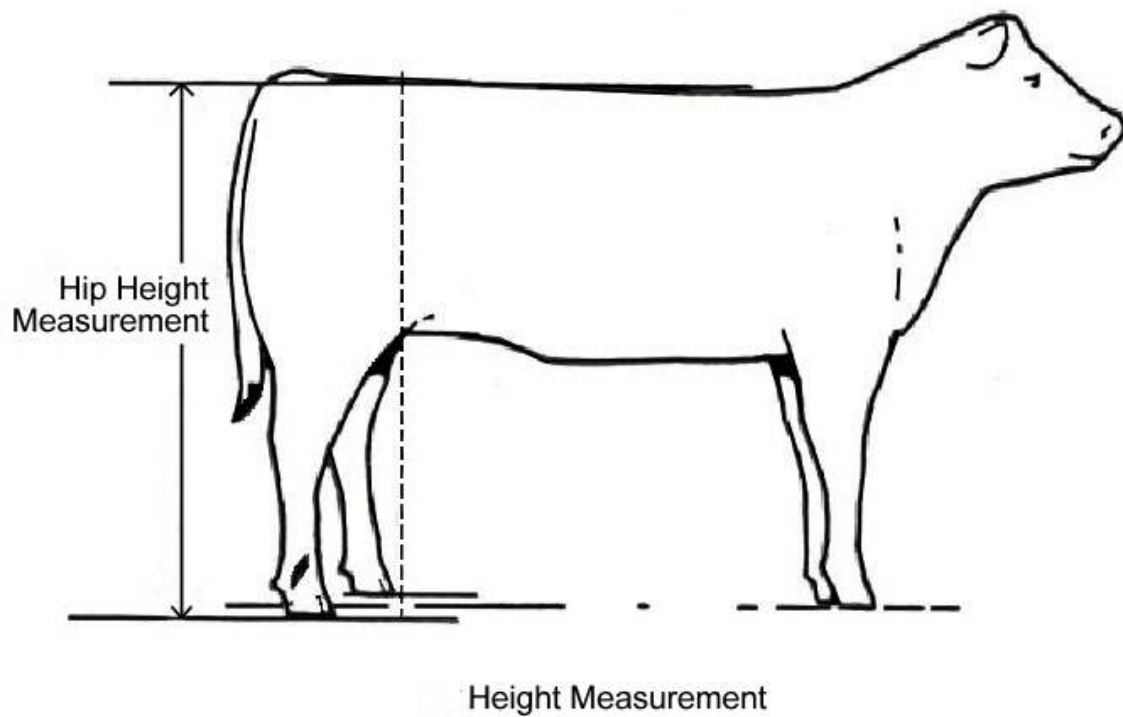


Figure 3.3. Drawing indicating the location for hip height measurement (BIF, 1990).

day. The heifers in the MGA group received the same ration supplement as the heifers in the Control group with the following exception; MGA (MGA 500 liquid premix; Pharmacia & Upjohn Company, Kalamazoo, MI) was added to the ration and fed for 14 days at a rate of 0.5 mg per head per day. The first MGA treatment began when the average age of the heifers reached ~10 months of age. The second MGA treatment began when the average age of the heifers was ~12 months of age and the third when the average age of the heifers was ~14 months of age. Each MGA treatment period was separated by 17 days with no MGA in the feed.

The heifers in the Rumensin group received the same ration supplement as the heifers in the Control group with the following exception, sodium monensin (Rumensin 80 premix; Elanco Animal Health Division of Eli Lilly and Company, Indianapolis, IN) was added to the ration and fed continuously from December 1st to April 14th. Sodium monensin was fed at an initial rate of 100 mg per head per day for 5 days and then increased to 200 mg per head per day for the remainder of the feeding period. The heifers in the MGA+Rumensin group were fed a ration that was a combination of the MGA and Rumensin treatments.

Starting with the initiation of the first MGA treatment (January 5th) estrual activity was monitored by Estrus Alert Patches (Estrus Alert, Western Point, Inc., Apple Valley, MN) checked once daily as well as visual estrus detection or mounting activity was monitored by the HeatWatch[®] System (DDx. Inc., Denver, CO) (Figures 3.4, 3.5, 3.6).

The ovaries of the heifers were examined via rectal palpation and transrectal ultrasonography (Aloka SSD 500 equipped with a 5.0 MHz linear array transducer; Corometrics Medical Systems, Wallingford, CT) following each 14-day MGA treatment/withdrawal and then continued for 9 or 10 days. Follicles were counted, measured and recorded for each ovary. Follicles that measured ≤ 5 mm were given a score of small, while follicles that measured 6 to 9 mm were given a score of medium and follicles measuring ≥ 10 mm were given a score of large (Lucy et al., 1990). Ovary scores were given for each ovary at every examination period via rectal palpation. Ovary scores were given based on a 1 to 5 scale, with a score of 1 being anestrus and a score of 5 being very active. The presence of a corpus luteum on the ovary was determined via palpation per rectum and verified by transrectal ultrasonography. Jugular vein blood samples were collected in 10 mL glass blood tubes containing



Figure 3.4. Estrus Alert Patches (Estrus Alert, Western Point, Inc., Apple Valley, MN) on the tail heads of crossbred beef heifers at Louisiana State University, Idlewild Experiment Station.



Figure 3.5. A HeatWatch® (DDx. Inc., Denver, CO) patch and transponder attached to the tail head of an experimental heifer at Louisiana State University, Idlewild Experiment Station.

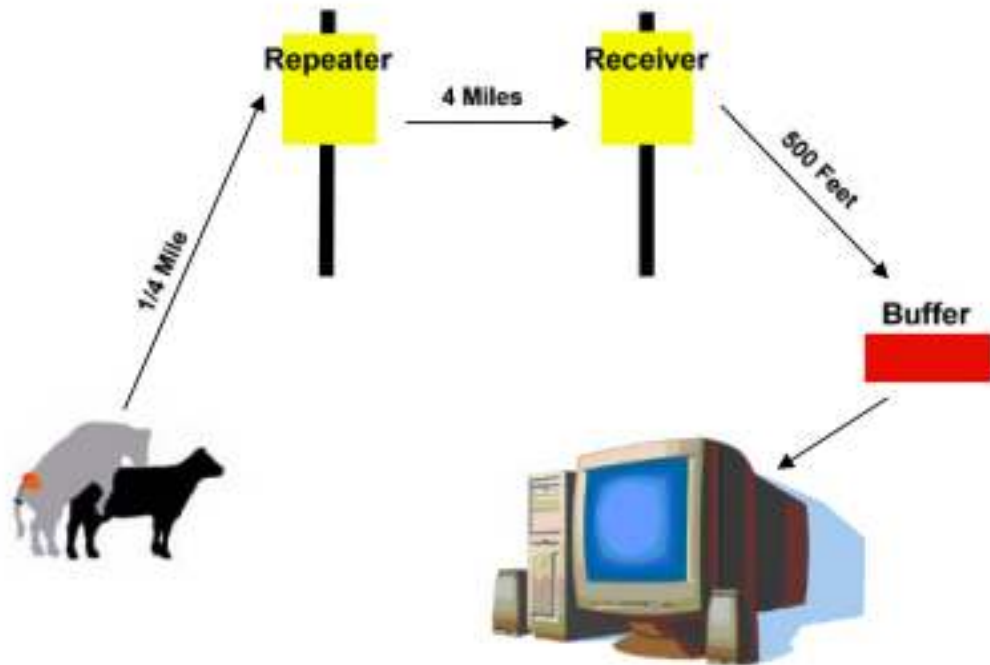


Figure 3.6. The HeatWatch[®] System (DDx. Inc., Denver, CO). The diagram illustrates the process of how the HeatWatch[®] System works (Diagram from DDx. Inc., Denver, CO).

sodium heparin at each ultrasound session and at 28-day intervals. All blood samples were centrifuged and the plasma was harvested and stored at -20°C to be assayed in the future for progesterone via radioimmunoassay (RIA).

Just prior to the start of the 65-day breeding season the heifers were allocated to two groups for breeding. One group was comprised of the Rumensin and MGA+Rumensin groups, while the other was comprised of the Control and the MGA groups. At the beginning of the breeding season, all heifers were subjected to two injections of prostaglandin-F_{2α} (Lutalyse[®] dinoprost tromethamine, Pharmacia & Upjohn Company, New York, NY) 11 days apart, to synchronize their estrous cycles, with the first injection given on April 7th 17 days following withdrawal of the last MGA treatment, and the 2nd injection given on April 18th. Following the second injection the heifers were monitored for estrual activity either via Estrus Alert Patches (Estrus Alert) and visual estrus detection checked twice daily or estrual standing activity using the HeatWatch[®] System. Responding heifers were artificially inseminated 12 to 18 h after the onset of standing estrus.

After 5 days into the breeding season, two fertile “clean-up” Angus bulls were placed with the heifers (one bull per group) for the remainder of the 65-day breeding season. Bull fertility was evaluated prior to the breeding season using a breeding soundness exam (BSE). Heifers that were artificially inseminated were placed with bulls 48 hours after the last standing estrus had been detected following the 5-day allotted estrus synchronization interval. The same two bulls were used for both replicates. There were 22-23 heifers per bull for Replicate 1 and 20 heifers per bull for Replicate 2. The bulls were rotated between groups 4 times during the 65 day restricted breeding season to reduce any bull effect.

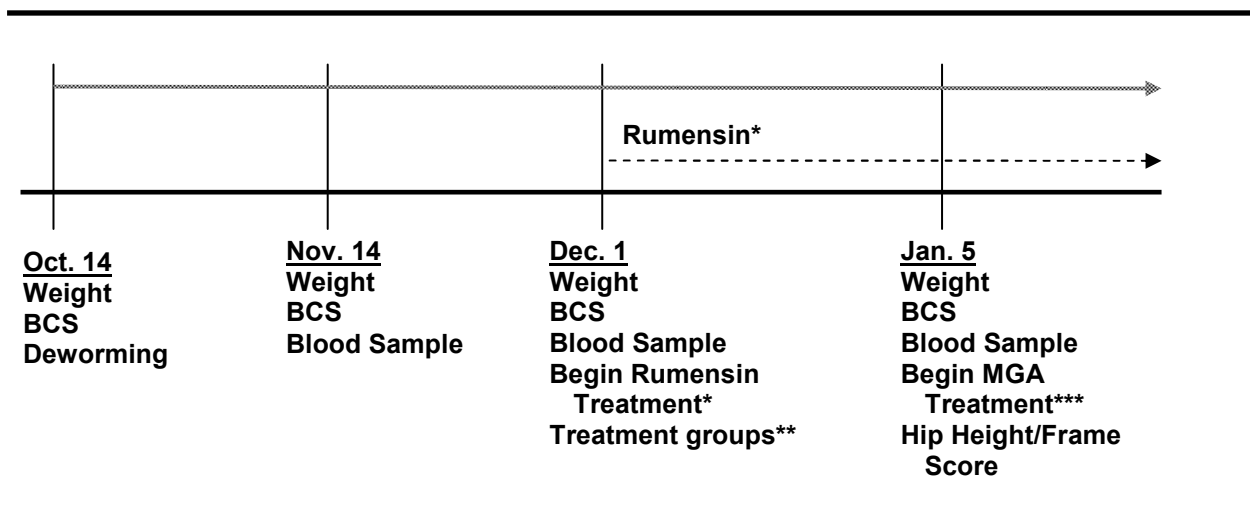
At 30 days after the end of the bull breeding season, all the heifers were palpated per rectum and ultrasonically examined for pregnancy determination. Fetal age was determined via ultrasonography utilizing crown-rump and head diameter measurements (Beal, 2000).

Due to the redundancy of some sampling times during Replicate 1, some minor adjustments concerning sampling dates were made for Replicate 2 after analyzing data from Replicate 1. The adjustments for Replicate 1 included estrual activity monitored by Estrus Alert Patches checked once daily combined with 1 hour of visual estrus detection

twice daily at 6:00am and 6:00pm. For Replicate 2, standing activity was monitored via the HeatWatch[®] System. For Replicate 1, a random sample was taken from the experimental animals for ultrasound examination of ovarian and follicular dynamics. The ovaries of 12 randomly selected heifers (3 per treatment group) were examined every 2 days via transrectal ultrasonography beginning the 3 days after each MGA treatment withdrawal and continued for 10 days. For Replicate 2, the ovaries of all 40 animals were examined every 3 days via transrectal ultrasonography (Aloka SSD 500 equipped with a 5.0 MHz linear array transducer) beginning 3 days after MGA treatment withdrawal and then continued for 9 days.

For Replicate 1, jugular blood samples were collected at each ultrasound examination for the 12 randomly selected heifers. This protocol was performed on the same 12 randomly selected heifers after each MGA treatment period. Jugular blood samples were also collected on all animals every 7 days to be processed for future progesterone assays via RIA. Jugular blood samples were collected in 10 mL glass blood tubes, containing sodium heparin, at each ultrasound session and at 28-day intervals for all animals during Replicate 2. A time line for Replicate 1 of the experiment is represented in Figures 3.7 and 3.8.

Also for Replicate 2, additional data were collected to be analyzed separately. Ovaries of all heifers were also examined via transrectal ultrasonography at the initiation of each MGA treatment and 7 days after initiation to assess the effectiveness of the MGA to suppress ovarian activity during the treatment period. Ovarian activity was also assessed for the Control and Rumensin groups. Reproductive tract scores were given to all females on the initiation of the experiment, as well as, prior to each MGA treatment period (Figures 3.9 and 3.10). Reproductive tract scores were given based on ovary score, ovarian activity and a uterine horn score. The uterine horn score was given on a 1 to 5 scale. A score of 1 is defined as prepubertal while a score of 5 would be mature. This technique is a variation of the method explained by Anderson (1987) (see Table 3.1). In addition, animals that responded to estrus synchronization and were artificially inseminated were palpated per rectum and ultrasonically examined for pregnancy determination 30 days post-insemination to verify pregnancy from artificial insemination. A time line of Replicate 2 of the experiment is represented in Figures 3.9 and 3.10.

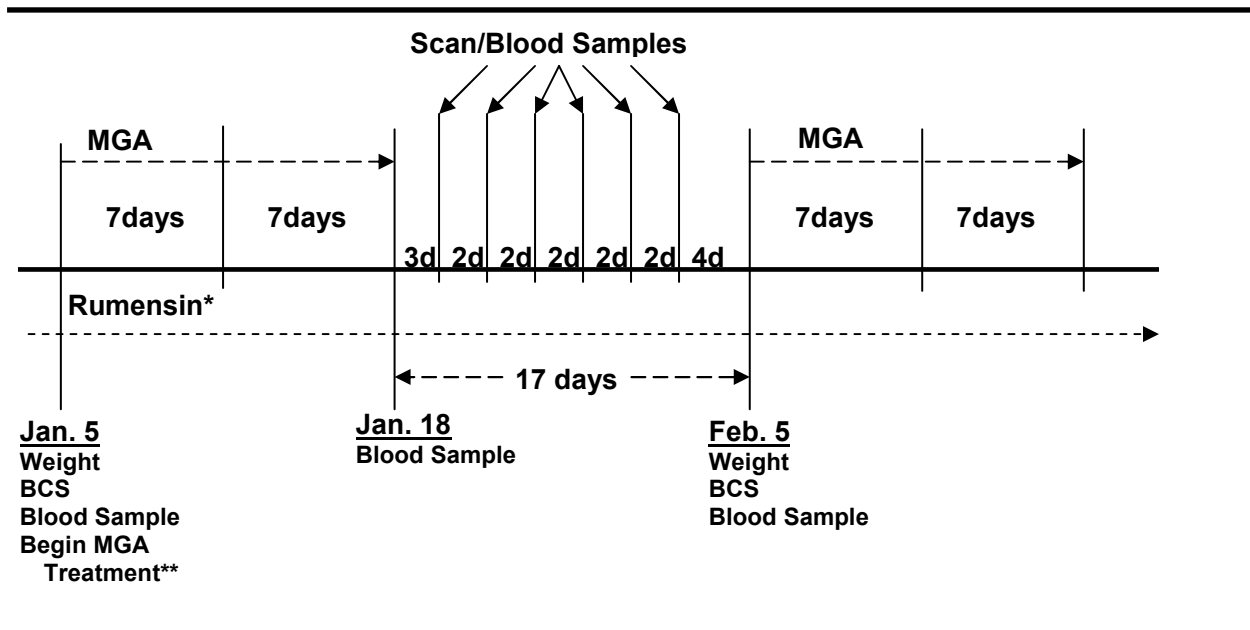


*Rumensin fed to groups Rumensin and MGA+Rumensin only at 100 mg/head/day for the first 5 days then 200 mg/head/day for the remainder of the treatment period.

**Forty-five heifers were stratified by adjusted weaning weight into four groups. Three groups of 11 animals and one group of 12 animals. Treatments were randomly assigned to the four groups. Treatments consisted of Control, MGA, Rumensin, and MGA+Rumensin combined.

***MGA fed to groups MGA and MGA+Rumensin only at 0.5 mg/head/day for 14 days in three separate treatments with 17 days between each treatment.

Figure 3.7. Sequence of events outlining the experimental procedure for Replicate 1.



*Rumensin fed continuously from December 1st until April 14th.

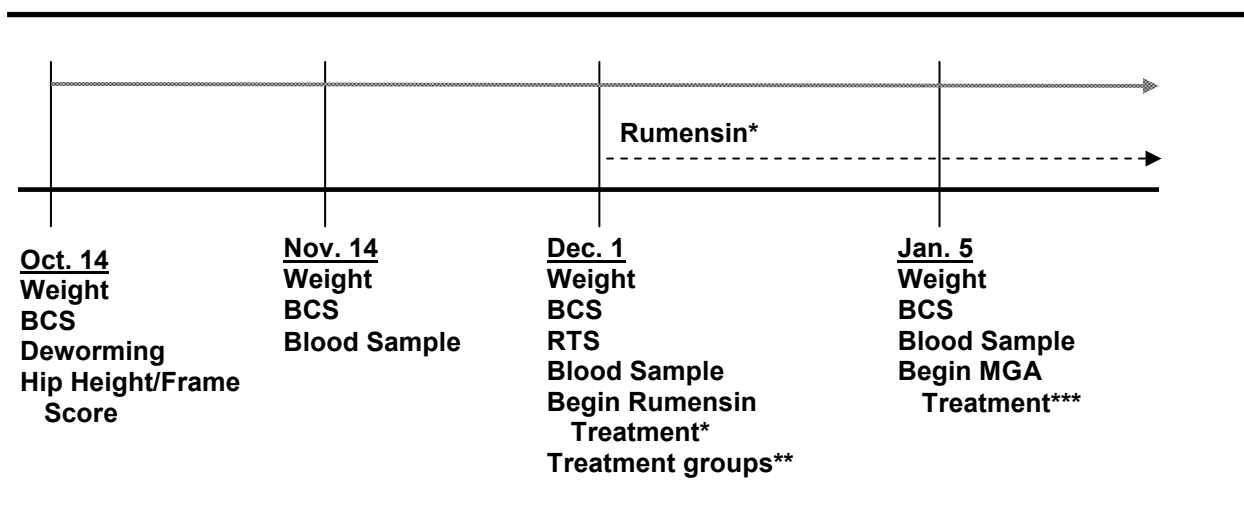
**MGA fed to groups MGA and MGA+Rumensin only at 0.5 mg/head/day for 14 days in three separate treatments with 17 days between each treatment. Time line representing two MGA treatment periods, which there were three treatment periods during the complete Replicate.

Figure 3.8. Continuation of the events outlining the experimental procedure for Replicate 1.

Table 3.1. Description of reproductive tract scores for Replicate 2 of the study.

Reproductive Tract Score	Uterine Horns	Approximate Ovary size	Ovarian Structures
1	Immature <20 mm diameter, no tone	15 mm X 10 mm X 8 mm	No palpable structures
2	20-25 mm diameter, no tone	18 mm X 12 mm X 10 mm	8 mm follicles
3	25-30 mm diameter, slight tone	22 mm X 15 mm X 10 mm	8-10 mm follicles
4	30 mm diameter, good tone	30 mm X 16 mm X 12 mm	>10 mm follicles, Corpus luteum possible
5	>30 mm diameter, good tone	>32 mm X 20 mm X 15 mm	>10 mm follicles, Corpus luteum present

The table was adapted from Anderson (1987).

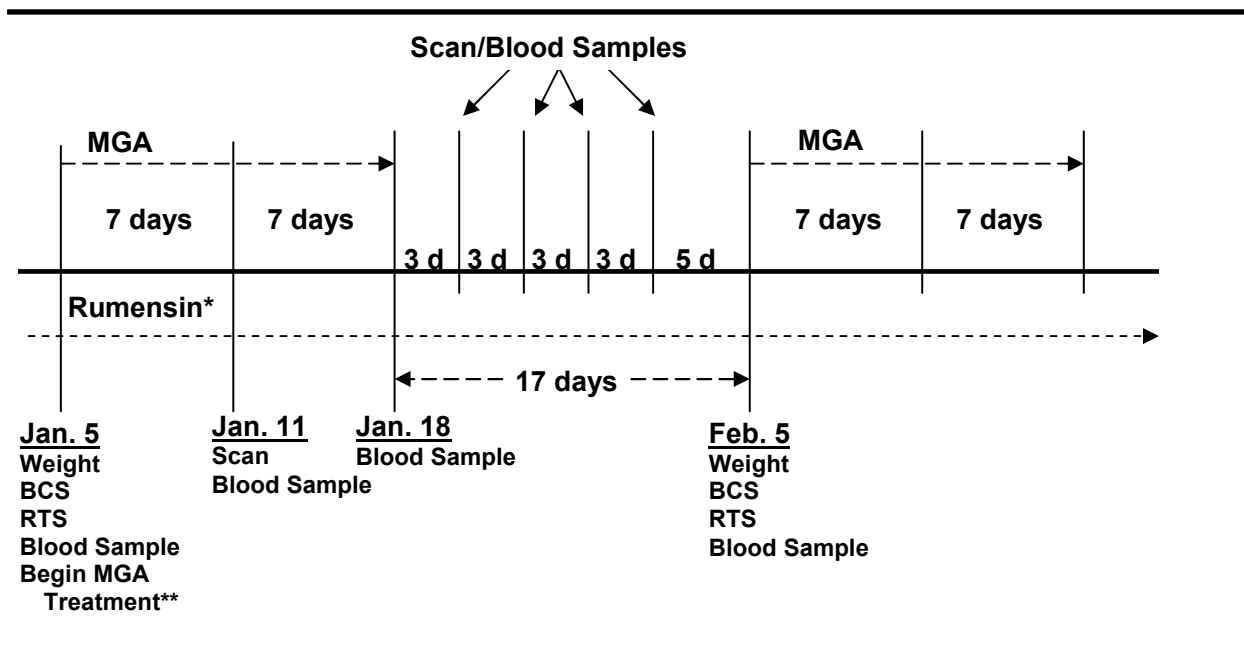


*Rumensin fed to groups Rumensin and MGA+Rumensin only at 100 mg/head/day for the first 5 days then 200 mg/head/day for the remainder of the treatment period.

**Forty heifers were stratified by adjusted weaning weight into four groups of ten animals. Treatments were randomly assigned to the four groups. Treatments consisted of Control, MGA, Rumensin, and MGA+Rumensin combined.

***MGA fed to groups MGA and MGA+Rumensin only at 0.5 mg/head/day for 14 days in three separate treatments with 17 days between each treatment.

Figure 3.9. Sequence of events outlining the experimental procedure for Replicate 2.



*Rumensin fed continuously from December 1st until April 18th.

**MGA fed to groups MGA and MGA+Rumensin only at 0.5 mg/head/day for 14 days in three separate treatments with 17 days between each treatment. Time line representing two MGA treatment periods, which there were three treatment periods during the complete Replicate.

Figure 3.10. Continuation of the events outlining the experimental procedure for Replicate 2.

Statistical Analysis

The experiment was arranged as a Randomized Block Design and was conducted over a 2-year period. To remove the effect of differences between years, the experiment was blocked by year, allowing data to be analyzed as replicates.

Treatment groups were compared using the least squares means for the variables birth weight by treatment, adjusted weaning weight by treatment, frame score by treatment, postweaning gain or loss by treatment, average daily gain throughout the experimental periods by treatment, breeding weight by treatment, breeding body condition score by treatment, change in body condition score throughout the experimental periods by treatment, observation of estrus prior to the breeding season by treatment and corpora lutea formation prior to the breeding season per animal by treatment, utilizing the two-way analysis of variance option in the General Linear Model procedure outlined in SAS Version 8 statistical software (SAS Institute, Inc., Cary, NC). Least squares means for the variable fetal age at pregnancy determination, for pregnant animals only, were compared using the two-way analysis of variance option in the General Linear Model procedure outlined in Sigma Stat statistical software (Systat Software, Inc., San Jose, CA).

Comparisons were made between treatment groups for the variable pregnancy status at 30 days postbreeding season by using the Chi-Square test in the Frequency Procedure outlined in SAS Version 8 statistical software. To remove the effect of differences between years, the experiment was blocked by year.

Treatment groups were compared using the least squares means for the variable ovary score by date, utilizing the analysis of variance option in the General Linear Model procedure outlined in SAS Version 8 statistical software. Replicates 1 and 2 were analyzed separately due to collection time variations between replicates.

Treatment groups, for Replicate 2 only, were compared using the least squares means for the variables breeding reproductive tract score and change in reproductive tract score throughout the experimental portion of Replicate 2, utilizing the analysis of variance option in the General Linear Model procedure outlined in SAS Version 8 statistical software. The results were compared using the Least Significant Difference (LSD) test in the General Linear Model procedure of SAS Version 8 statistical software.

Results

The data collected for Replicates 1 and 2 were continuous with a few exceptions that are noted in this chapter. Any unrelated data set collected for Replicates 1 and 2 was analyzed separately. The experimental design was the same for Replicates 1 and 2, which enabled most data from both replicates to be combined. Because the experimental animals were beef animals, the data were reported under three categories consisting of performance data, breeding data, ovary and follicle data.

Animal Performance Data

Animal performance data are summarized in Table 4.1. These data were combined for Replicates 1 and 2 and are reported as an average per animal per treatment group for both replicates ($n = 85$). There were no significant differences detected ($P > 0.05$) among the four treatment groups for average frame score, average birth weight, average adjusted weaning weight, average postweaning gain or loss in weight, average daily gains, average breeding weight, average breeding body condition score (BCS), or average change in BCS throughout the experimental periods.

Ovary and Follicle Data

Ovary scores given at each heifer examination were analyzed as average ovary scores per female over time by treatment. There was a significant interaction ($P < 0.05$) among treatment groups for the change in size of the left ovary over the course of both replicates. Average ovary scores over time by treatment group for Replicates 1 and 2 of the experiment are shown in Figures 4.1 and 4.2 respectively.

Follicle data and ovary scores over time were analyzed separately for each replicate due to the difference in examination days and the time between examinations. Follicle measurements were performed via ultrasonography. Follicles measured during an examination were determined to be small, medium or large in size. A small follicle score was given if the follicle size was smaller than 6 mm. A medium follicle score was given if the follicle size was 6 to 9 mm while a large follicle score was given if the follicle measurement was 10 mm or greater (Lucy et al., 1990). Follicle data for Replicate 1 of the experiment is summarized in Table 4.2. Follicle data for Replicate 2 of the study are summarized in Table 4.3.

During the treatment period for Replicate 1 of the experiment, no significant differences ($P>0.05$) were detected among treatment groups for the average number of medium follicles (6 to 9 mm) per animal per examination (Table 4.2).

During the treatment period for Replicate 1 of the experiment, significant differences ($P<0.05$) were detected among treatment groups ($n = 3$ animals per group) for the average number of large follicles (≥ 10 mm) per animal per examination (Table 4.2). The Rumensin group developed significantly ($P<0.05$) more large follicles per animal per examination than the other three treatment groups. The MGA and MGA+Rumensin groups also developed significantly ($P<0.05$) more large follicles per animal per examination than the Control group. There was no significant difference ($P>0.05$) detected for average number of large follicles per animal per examination between MGA and MGA+Rumensin groups.

During the treatment period for Replicate 2 of the experiment, significant differences ($P<0.05$) were noted among treatment groups ($n = 10$ per group) for the average number of medium (6 to 9 mm) follicles per animal per examination (Table 4.3). The Control and Rumensin groups had significantly ($P<0.05$) more medium follicles per animal per examination than the MGA+Rumensin or MGA groups. No significant differences ($P>0.05$) were detected for the average number of medium follicles observed per animal per examination between the Control and Rumensin groups or between the MGA group and MGA+Rumensin groups.

During the treatment period for Replicate 2, significant differences ($P<0.05$) were detected among treatment groups for the average number of large follicles (≥ 10 mm) per animal per examination (Table 4.3). The MGA group had significantly ($P<0.05$) more large follicles per animal per examination than any of the other treatment groups. The MGA+Rumensin and the Rumensin groups also had significantly ($P<0.05$) more large follicles per animal per examination than the Control group. No significant difference ($P>0.05$) was detected between the MGA+Rumensin group and the Rumensin group for the average number of large follicles per animal per examination.

Breeding Data

For breeding data, the average number of corpora lutea formed per animal per group during the breeding season was combined for Replicates 1 and 2 and is summarized in Table 4.4. No significant differences ($P>0.05$) were detected among

treatment groups for the average number of corpora lutea formed per animal per group, prior to the breeding season.

Reproductive tract scores (RTS) were only implemented during Replicate 2 and therefore only analyzed for Replicate 2 on an average RTS per group. RTS data were also analyzed for the change in RTS throughout the experimental period for Replicate 2. RTS data are summarized in Table 4.5. The artificial insemination pregnancy percentages were only recorded for Replicate 2 and due to the low number of animals responding to estrous synchronization per group, were not statistically analyzed. The artificial insemination pregnancy percentages for Replicate 2 are also reported in Table 4.5.

There were significant differences ($P < 0.05$) detected among treatment groups for average breeding RTS per group and average change in RTS per group throughout the experimental period for Replicate 2 (Table 4.5). The MGA+Rumensin group had significantly ($P < 0.05$) larger reproductive tract scores at the time of breeding than both the Rumensin group and the Control group. The MGA+Rumensin group also had a significantly ($P < 0.05$) greater change in RTS throughout the experimental period during Replicate 2 than either the Rumensin or Control groups. There were no significant differences ($P > 0.05$) detected for average breeding RTS or change in RTS throughout the experimental period for Replicate 2 among the Rumensin, MGA or Control groups. There was also no significant difference detected between the MGA+Rumensin group and the MGA group for these variables ($P > 0.05$).

The average number of animals that exhibited estrual behavior per group, the pregnancy percentage from artificial insemination in conjunction with natural mating, and average fetal age at pregnancy determination were combined and analyzed for Replicates 1 and 2. These data are summarized in Table 4.6. The average number of animals that responded to estrous cycle synchronization per group is also shown in Table 4.6, but was not analyzed due to the low number of animals responding to the estrous synchronization treatment.

There were no significant differences ($P > 0.05$) detected among treatment groups for the average number of animals observed exhibiting estrual behavior during the experimental periods (Table 4.6). Among the animals responding to estrous synchronization, the MGA and MGA+Rumensin groups had a 57% and 41% response

rate, respectively. The Rumensin and Control groups had a 5% and 10% response rates, respectively.

There were notable significant differences ($P < 0.05$) among treatment groups for pregnancy percentage (Table 4.6). The Rumensin and MGA+Rumensin groups had significantly ($P < 0.05$) greater pregnancy percentages, 95% (20/21) and 91% (20/22) respectively, from artificial insemination or natural mating during the 65-day synchronized breeding season than that of the Control group having a 61% (13/21) pregnancy percentage. The Rumensin group alone had a higher ($P < 0.05$) pregnancy percentage, at 95% (20/21), than that of the MGA group, having a 71% (15/21) pregnancy percentage. There were no significant differences ($P > 0.05$) noted between the Control group and the MGA group, the MGA+Rumensin group and the MGA group, or the MGA+Rumensin group and the Rumensin group.

There were no significant differences ($P > 0.05$) noted among treatment groups for the average fetal age at a 30-day postbreeding season pregnancy evaluation (Table 4.6). On average, all heifers that were determined to be pregnant became so during the first 21 days of a synchronized 65-day breeding season.

Discussion

Selection and a high plane of nutrition are the basis for obtaining early maturing replacement heifers and should always take priority, but there must be a balance between selection for beef production and selection for fertility. Without these basic management considerations, it is very probable that the fertility of replacement animals will be unchanged or reduced. Though selection is the basis for obtaining early maturing animals, it should not be so radical, as to reduce the production of beef to supplement fertility. Therefore, when the nutrient requirements are met for a group of replacement beef heifers there is still a need for management practices that will further increase the fertility of replacement beef heifers during their first breeding season. In this study, the nutrient requirements were met for all animals and stratification across groups was implemented instead of selection. All of the experimental animals were crossbred animals sired by the same bulls for both replicates, therefore the genetic influences and nutritional effects were held relatively consistent across treatment groups, which leave treatments as the primary influence.

Table 4.1. Combined performance data for heifers during Replicate 1 and Replicate 2. These data are reported on an average per animal per treatment group for both replicates combined.

Treatment	N	Avg. Birth Weight (kg)**	Avg. Adj. Weaning wt. (kg)**	Avg. Frame Score*	Avg. Gain/Loss		ADG (kg)**	Avg. Breeding Wt. (kg)**	Avg. Breeding BCS	Change in BCS***
					Postweaning (kg)**					
Control	21	34.1 ± 1.68	185.0 ± 3.56	4.81 ± 0.21	5.70 ± 1.19		0.50 ± 0.03	296.0 ± 6.30	5.48 ± 0.14	0.38 ± 0.18
MGA	21	37.7 ± 1.68	191.6 ± 3.56	5.25 ± 0.21	7.16 ± 1.19		0.50 ± 0.03	305.7 ± 6.30	5.52 ± 0.14	0.53 ± 0.18
Rumensin	21	36.9 ± 1.68	191.1 ± 3.56	5.28 ± 0.21	7.92 ± 1.19		0.58 ± 0.03	317.1 ± 6.30	5.82 ± 0.14	0.70 ± 0.18
MGA+Rum	22	35.8 ± 1.65	193.1 ± 3.49	5.03 ± 0.20	8.55 ± 1.16		0.53 ± 0.03	309.4 ± 6.18	5.66 ± 0.13	0.64 ± 0.17

*Frame score formula calculated from Dhuyvetter (1995).

**Weights obtained every 28 to 30 days during the experiment. Weight documented at the beginning of the heifer breeding season was used to calculate the average breeding weight.

***Change in body condition score measured from beginning to end of each replicate.

^{a,b,c}Different superscripts represents a difference (P<0.05) among treatment groups within columns.

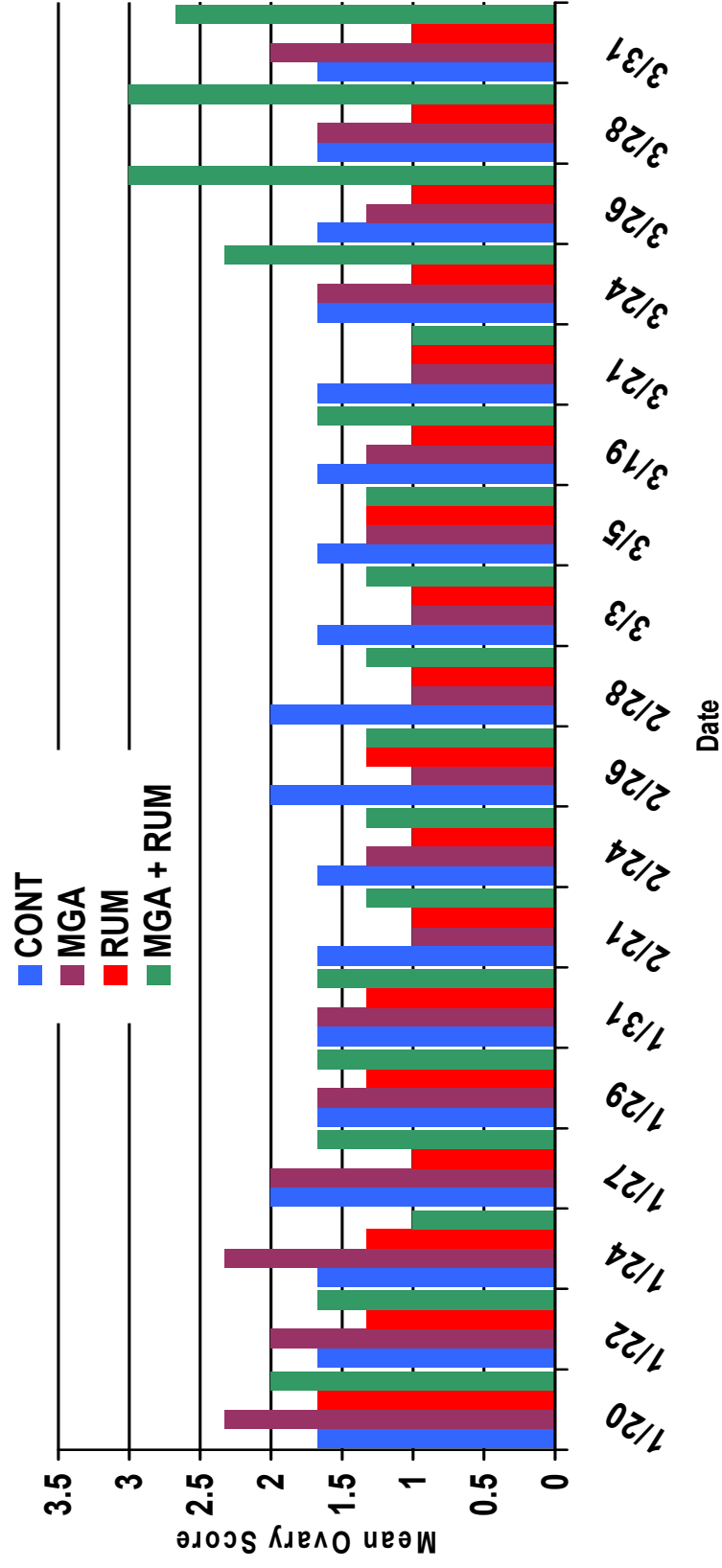


Figure 4.1. Mean ovary scores for heifers over time by treatment group for Replicate 1 (n = 3 heifers per group). Only the left ovaries are shown. Only the left ovaries for Replicate 1 heifers showed a significant interaction ($P < 0.05$) among groups for treatment over time.

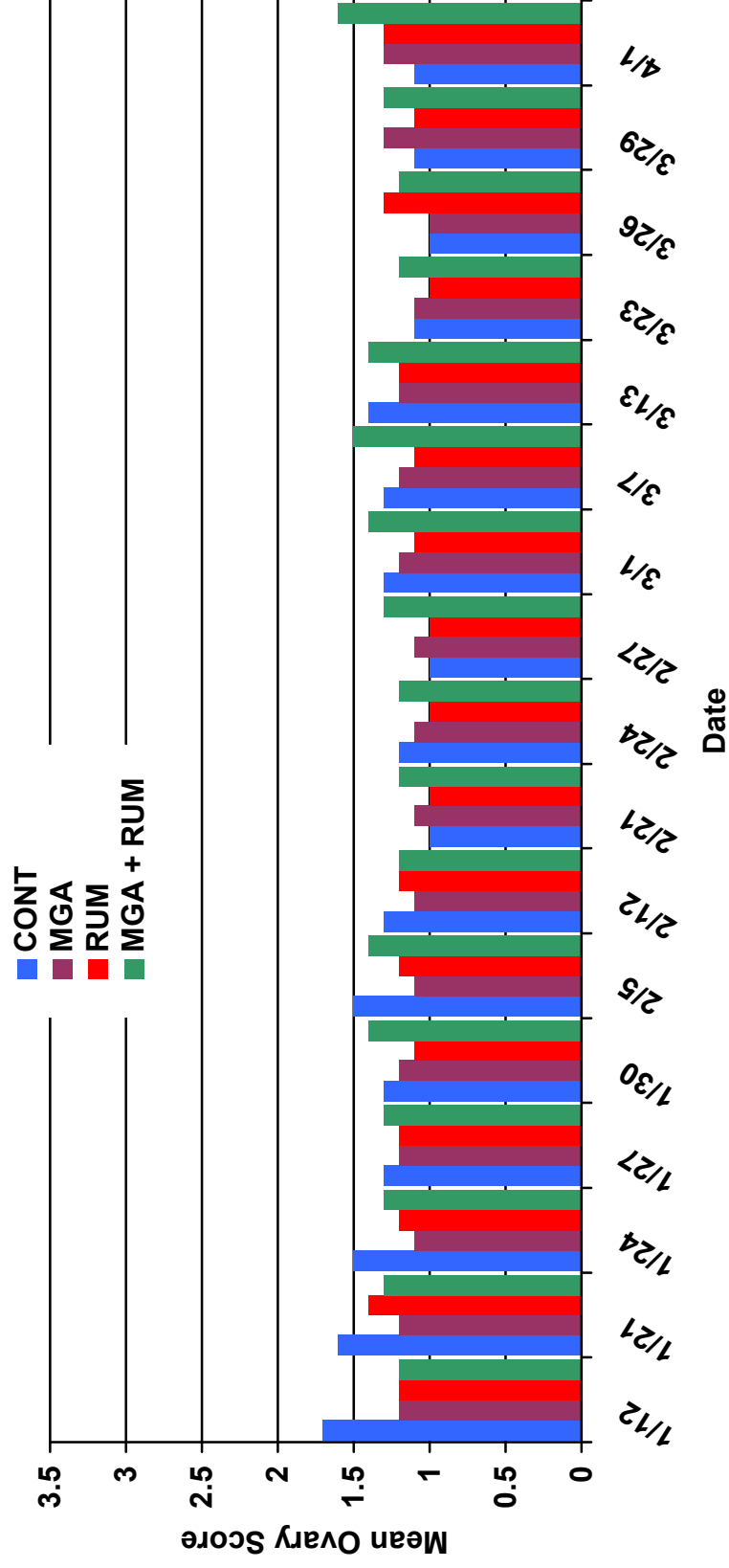


Figure 4.2. Mean ovary scores for heifers over time by treatment group for Replicate 2 (n = 10 heifers per group). Only the left ovaries are shown. Only the left ovaries for Replicate 2 heifers showed a significant interaction ($P < 0.05$) among groups for treatment over time.

Table 4.2. Average number of medium and large follicles per animal per examination during the Replicate 1 experimental period.

Treatment Group	n*	Medium Follicles**	Large Follicles***
Control	3	1.30	0.28 ^a
MGA	3	1.15	0.65 ^b
Rumensin	3	1.33	0.96 ^c
MGA+Rum	3	1.13	0.72 ^b
SEM****		± 0.24	± 0.11

*Three animals per treatment palpated per rectum 18 times each with follicular diameter measured via ultrasonography. Follicular dynamics for Replicate 1 were recorded six times (every 2 days over a period of 17 days) between the three MGA treatment periods and after the last MGA treatment.

**Medium follicle diameter between 6-9 mm (Lucy et al., 1990).

***Large follicle diameter between ≥10 mm (Lucy et al., 1990).

****SEM = Standard error of the mean.

^{a,b,c} Different superscripts represents a difference (P<0.05) among treatment groups within columns.

Table 4.3. Average number of medium and large follicles per animal per examination during the Replicate 2 experimental period.

Treatment Group	n*	Medium Follicles**	Large Follicles***
Control	10	2.62 ^a	0.28 ^a
MGA	10	2.09 ^b	0.69 ^b
Rumensin	10	2.49 ^a	0.51 ^c
MGA+Rum	10	2.11 ^b	0.54 ^c
SEM****		± 0.16	± 0.09

*Ten animals per treatment palpated per rectum 12 times each with follicular diameter measured via ultrasonography. Follicular dynamics for Replicate 2 were recorded four times (every 3 days for the first 12 days of the 17-day interval between treatments) between the three MGA treatment periods and after the last MGA treatment.

**Medium follicle diameter between 6-9 mm (Lucy et al., 1990).

***Large follicle diameter between ≥10 mm (Lucy et al., 1990).

****SEM = Standard error of the mean.

^{a,b,c} Different superscripts represents a difference (P<0.05) among treatment groups within columns.

Table 4.4. Average number of corpora lutea formed per animal per group during the experimental periods for Replicates 1 and 2 combined.

Treatment	n*	Avg. Number of CL Formation Prior to Breeding Season**
Control	13	0.90
MGA	13	1.28
Rumensin	13	0.55
MGA+Rum	13	1.65
SEM***		± .50

*Number of animals represents the 12 animals randomly selected from Replicate 1 (3 per treatment group) and all animals from Replicate 2 (10 per treatment group).

**Mean number of CL per animal per treatment group present during rectal palpation and visualized via ultrasonography.

***SEM = Standard error of the mean.

^{a,b,c} Different superscripts represent a difference ($P < 0.05$) among treatment groups within columns.

Table 4.5. Breeding data for Replicate 2 only. Results for reproductive tract score (RTS) are given as the mean per group.

Treatment	n	Avg. Breeding RTS* (Rep 2)	Avg. Change in RTS** (Rep 2)	AI Pregnancy Rate (Rep 2)	Natural Mating Pregnancy Rate (Rep 2)
Control	10	2.2 ± 0.43 ^a	+1.2 ± 0.43 ^a	2/2 (100%)	6/8 (75%)
MGA	10	2.5 ± 0.43 ^{a,b}	+1.5 ± 0.43 ^{a,b}	4/5 (80%)	4/6 (67%)
Rumensin	10	2.0 ± 0.43 ^a	+1.0 ± 0.43 ^a	0/1 (0%)	10/10 (100%)
MGA+Rum	10	3.0 ± 0.43 ^b	+2.0 ± 0.43 ^b	3/3 (100%)	7/7 (100%)

*Mean reproductive tract score given at the start of the 65-day breeding season for Replicate 2.

**Mean numerical change in reproductive tract score, given prior to the start of the experimental period for Replicate 2 and the last given at the start of the synchronized 65-day breeding season for Replicate 2.

^{a,b,c} Different superscripts represents a difference (P<0.05) among treatment groups within columns.

Table 4.6. Combined breeding data for Replicate 1 and Replicate 2.

Treatment	n	Avg. No. Estrus Obsr*	Response to Synchronization w/ PGF2 α	Pregnancy % AI and Natural Mating	Avg. Fetal Age** (days)
Control	21	1.10 \pm .30	2/21 (10%)	13/21 (61%) ^a	74.2 \pm 4.6
MGA	21	0.92 \pm .30	12/21 (57%)	15/21 (71%) ^{a,c}	82.7 \pm 4.2
Rumensin	21	0.51 \pm .30	1/21 (5%)	20/21 (95%) ^b	76.4 \pm 3.6
MGA+Rum	22	0.91 \pm .29	9/22 (41%)	20/22 (91%) ^{b,c}	86.7 \pm 3.6

*Estrual behavior was recorded visually and by the use of Estrus Alert patches during Replicate 1. Replicate 2 estrual behavior was recorded by the use of the HeatWatch[®] System. Estrual behavior is reported as the average number of estrual observations per female per treatment group.

**Fetal age was determined via ultrasonography using crown-rump and fetal head diameter measurements. Days are in respect to a 30 day postbreeding season pregnancy evaluation (Beal, 2000).

^{a,b,c} Different superscripts represents a difference (P<0.05) among treatment groups within columns.

Though not statistically significant, the control animals were the lightest and smallest framed animals in this experiment. There was an 8.13 kg difference in adjusted weaning weight between the heaviest group and the lightest group, but this difference was not statistically significant ($P>0.05$). The explanation for this is only random selection. The treatments were randomly applied to the stratified groups. Though differences among groups prior to the start of the experiment should have been eliminated by stratification, a few individual animals that were much smaller than their counterparts may have skewed the mean adjusted weaning weights in spite of stratification. The author believes that these discrepancies did not affect the outcome of this experiment as these differences did not remain consistent across other variables analyzed for performance.

Though there were no significant differences among treatment groups for body condition score, average daily gains, average frame score, or breeding weight, body composition could have played a major role in the attainment of puberty of heifers in this experiment. It has been reported that increased body fat in young girls decreases age at puberty, which supports the role that leptin may have in age at puberty and fertility (Dunger et al., 2005; Kaplowitz, 2008). Though not determined in this experiment, it is a plausible explanation that animals fed sodium monensin had a higher ratio of body fat to muscle when compared with other treatment groups providing a rationale for the results documented in this experiment.

During Replicate 1 and Replicate 2, ovary scores were given to each ovary in each heifer at each heifer examination. For both replicates, a significant interaction was only noted among treatment groups for the mean ovary score per animal per treatment for the left ovary. The information provided by these data is that there were differences in the mean ovary score of the left ovary and the treatment group with the highest and lowest value changed dependent on the date of the examination. The interesting detail is that only the size of the left ovary was found to differ between groups over time. One possible explanation for this result is that the left ovary in pubertal beef heifers may have greater follicular activity than the right ovary.

In this study, the average number of medium and large follicles per animal per examination were analyzed separately for Replicate 1 and Replicate 2 due to the

implementation of a different ultrasound scanning schedule and a difference in the number of animals being scanned for follicle populations. These adjustments did not affect the ability of this project to be conducted as replicates for other variables nor did these adjustments affect the outcome of the experiment.

During Replicate 1, there were no significant differences among treatment groups for the average number of medium follicles (6-9 mm in diameter) per animal per examination. On average, the group fed only sodium monensin (the Rumensin group) developed significantly more large follicles (≥ 10 mm in diameter) than any of the other three treatment groups (Rumensin $\bar{x} = 0.96$ vs. Control $\bar{x} = 0.28$, MGA $\bar{x} = 0.65$, MGA+Rumensin $\bar{x} = 0.72$). Both the MGA+Rumensin and MGA groups had animals that developed significantly more large follicles per animal per examination than the control group. This information tends to be contradictory to the scientific literature stating that melengestrol acetate can increase large follicle development following its withdrawal (Hill et al., 1971; Smith and Day, 1990). However, due to the low number of animals scanned for follicle populations in this study, no inference can be made at this time. Nevertheless, on average, all treatment groups developed more large follicles than the heifers in the control group. These data are consistent with other studies reporting that MGA or monensin can increase large follicle development over that of untreated animals (Bushmich et al., 1980; Hill et al., 1971; Ortuno and Carson, 1985; Smith and Day, 1990).

For Replicate 2, the Control and Rumensin groups (Control $\bar{x} = 2.62$, Rumensin $\bar{x} = 2.49$) developed significantly more medium follicles (6-9 mm in diameter) than the MGA+Rumensin group or the MGA group (MGA+Rumensin $\bar{x} = 2.11$, MGA $\bar{x} = 2.09$), but the MGA+Rumensin and MGA groups had no significant difference between them. For large follicles (≥ 10 mm in diameter), the MGA group developed significantly more large follicles than the other treatment groups (MGA $\bar{x} = 0.69$ vs. Control $\bar{x} = 0.28$, Rumensin $\bar{x} = 0.51$, MGA+Rumensin $\bar{x} = 0.54$). In addition, the Rumensin and the MGA+Rumensin groups developed significantly more large follicles than the Control group. Data from Replicate 2 are in agreement with the scientific literature stating that

melengestrol acetate can increase large follicle development following its withdrawal (Hill et al., 1971; Smith and Day, 1990).

On average, all treatment groups in Replicate 2 of the experiment developed more medium sized follicles (6-9 mm in diameter) than the treatment groups in Replicate 1 of the experiment. The average number of large follicles (≥ 10 mm in diameter) per animal per examination remained relatively consistent for each treatment across years. It has been reported that short term increases in nutrition can stimulate positive changes in the numbers of small follicles that are recruited to a follicular wave that subsequently grow to medium sized follicles, but nutrition does not participate in follicle selection for dominance (Gutierrez et al., 1997). It is the author's belief that the differences in average number of medium sized follicles per animal per examination between years is due to a discrepancy in the level of nutrition available to the experimental animals between years, independent from the ration supplement provided. This difference in the level of nutrition between years is most likely due to environmental factors such as differences in quality of hay between years and the amount of available forage due to differences in rainfall between years.

In Replicate 1 of the experiment, MGA failed to enhance large follicle development above that of the other treatment groups. The reason for this is unknown, but it is possible that the difference in examination days post-MGA treatment between replicates could have played a role in the different results obtained between the replicates.

The results of this experiment revealed no significant additive effect of MGA treatments, alone or when combined with sodium monensin feeding, on the average number of corpora lutea formed per animal per group prior to the breeding season. Though not significant, groups receiving MGA formed more CL throughout the experiment than the control or Rumensin groups (MGA+Rumensin $\bar{x} = 1.65$, MGA $\bar{x} = 1.28$ vs. Control $\bar{x} = 0.90$, Rumensin $\bar{x} = 0.55$). The low number of CL developing per animal per group may have suppressed the ability of this experiment to show an additive effect of MGA on CL formation. Comparatively, this information is important because one functional CL could make a major difference in the maturational status of a prepubertal or peripubertal animal.

Unfortunately, all plasma samples to be analyzed for progesterone that were harvested for both replicates of the experiment were destroyed during a power outage and information that could have been gained from these samples is lost. Though the serum samples could have given more insight into the hormonal aspects of puberty in cattle, the information given by the data obtained from this experiment are of value from an applied as well as research standpoint.

Although, reproductive tract scores (RTS) were only implemented during the second replicate of the study, only the MGA+Rumensin treatment had a significantly greater mean breeding RTS per treatment group (MGA+Rumensin \bar{x} = 3.0 vs. MGA \bar{x} = 2.5, Control \bar{x} = 2.2, Rumensin \bar{x} = 2.0) and a greater change in mean RTS per treatment group throughout the experiment (MGA+Rumensin \bar{x} = 1.2 vs. MGA \bar{x} = 1.5, Control \bar{x} = 1.2, Rumensin \bar{x} = 1.0) when compared with the control group, suggesting a possible additive effect of the two treatments combined for RTS.

Reproductive tract scores do grossly assess uterine maturation in terms of growth, but they do not assess the uterine maturation at the cellular level, especially at the level of the endometrium. Therefore, another rationale for the results of this experiment is increased uterine maturation from feeding sodium monensin. It has been proposed that after puberty, physiological maturation of the uterus in young dairy heifers continues (Del Vecchio et al., 1992b; Swanson et al., 1972). This information suggests that, though all other aspects of adult fertility are present, the uterine environment could be dysfunctional and inadequate to maintain a pregnancy, therefore, decreasing chances of early embryo development and maintenance of pregnancy. The information supplied by this experiment justifies further research in the area.

In this study, three treatments with MGA prior to the breeding season did not increase the ability of crossbred beef heifers to become pregnant during a synchronized 65-day breeding season (MGA = 71% pregnancy rate vs. Control = 61% pregnancy rate). Subsequently, no additive effect of MGA when administered with monensin was identified. This information is in contrast with other studies indicating that treatment of pubertal heifers with an exogenous progestogen can increase pregnancy percentages (Ghallab et al., 1984; Gonzalez-Padilla et al., 1975b; Jaeger et al., 1992). Gonzalez-Padilla et al. (1975b) were among the first groups to demonstrate the efficacy of

exogenous progestogens to induce the onset of puberty in heifers while increasing pregnancy percentage. Ghallab et al. (1984) used repetitive norgestomet implants (a strong synthetic progestogen) to induce puberty in heifers while increasing their pregnancy percentage. Jaeger et al. (1992) used melengestrol acetate in conjunction with PGF_{2α} to effectively induce puberty in beef heifers and increase their pregnancy percentage. They noted that MGA+PGF_{2α} treatment not only induced puberty in Simmental, Angus and Hereford crossbred heifers but effectively synchronized estrous cycles and enabled a higher percentage (49% treated vs. 14% untreated) of animals to become pregnant early in the breeding season. To date, the researchers in this experiment have no knowledge of using multiple treatments of the oral progestogen, MGA, to induce puberty in prepubertal heifers and assist them through multiple estrous cycles prior to the breeding season.

This study did reveal the capability of monensin to increase the pregnancy rate of crossbred beef heifers during a synchronized 65-day breeding season (Rumensin with a 95% pregnancy rate, MGA+Rumensin with a 91% pregnancy rate vs. MGA with a 71% pregnancy rate controls with a 61% pregnancy rate). These results are in agreement with various other reports that demonstrate the ability of sodium monensin to decrease age at puberty and increase fertility, though most studies have associated these effects with the alteration of ruminal fermentation toward ruminal propionate when fed sodium monensin (Baile et al., 1982; Lalman et al., 1993; McCartor et al., 1979; Meinert et al., 1992; Moseley et al., 1982; Purvis and Whittier, 1996). There is evidence suggesting that monensin may affect gonadotropin production by direct or indirect influence on the ovary and the hypothalamic-pituitary axis (Bushmich et al., 1980; Randel and Rhodes, 1980; Randel et al., 1982). These hormonal effects of feeding sodium monensin to cattle could have influenced the results of this experiment.

Armstrong and Spears (1988) were the first to provide evidence of the effect of monensin on ruminant metabolism independent of ruminal alterations. They achieved this through intravenous administration of monensin to beef heifers. These researchers reported contrasting results, concluding that intravenous administration of monensin suppressed LH release. Nevertheless, they provided evidence that monensin can directly affect serum concentrations of insulin, fatty acids, potassium, magnesium,

phosphorus and LH, independent of ruminal alterations. The researchers in this experiment have no knowledge of previous studies comparing the increase in fertility of prepubertal and peripubertal beef heifers when fed sodium monensin with similar animals that had been treated multiple times with MGA. There is also no knowledge of previous studies analyzing the additive effect of multiple treatments with MGA in combination with feeding monensin on fertility of beef heifers.

There are a few plausible explanations for the results of this experiment, which need further investigation to discover the physiological pathways in which sodium monensin plays a role in fertility of prepubertal and peripubertal heifers, regardless of the nutritional aspects of ionophore feeding described in the scientific literature. Further research is needed in the areas of hormone profiles of these animals, body composition and the physiological role that uterine maturation plays in conception and the maintenance of pregnancy.

When this experiment was conducted over two breeding seasons, there were no significant differences, on a per treatment group basis, for average frame score, birth weight, adjusted weaning weight, postweaning gain or loss, daily gains, breeding weight, breeding body condition score or the change in body condition score over the course of the treatment periods. This information indicates that the results documented during this experiment are a primary result of the treatments. The results of this experiment show that sodium monensin, when added to the diet of prepubertal and peripubertal crossbred beef heifers, can significantly increase the percentage of animals pregnant during their first breeding season (Rumensin at 95% pregnancy rate, MGA+Rumensin at 91% pregnancy rate vs. MGA at 71% pregnancy rate and the controls at 61% pregnancy rate). However, the method by which this occurs is not completely understood.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Managing replacement beef heifers to calve at 2 years of age could be a profitable management consideration for a beef producer. Heifers that are mated and conceive early within their first restricted breeding season will continue to calve earlier and wean heavier calves throughout their lifetime than those who conceive later (Lesmeister et al., 1973). Not only will calving at 2 years of age increase the value of the individual animal but it will increase the value of the herd. The calves from these heifers will be heavier and older at weaning than its counterparts that were conceived later in the breeding season.

The experiment described in this thesis was designed to examine the effects that multiple oral progestogen treatments had on fertility of prepubertal and peripubertal crossbred beef heifers while comparing progestogen priming to the feeding of sodium monensin from prepuberty to the breeding season. The experiment also examined the additive effects of using multiple progestogen treatments prior to the breeding season in combination with the feeding of sodium monensin. The goal of this experiment was to enhance the fertility of replacement heifers at the time of breeding, prior to 15 months of age, and to obtain a high percentage of animals pregnant that had conceived early in the breeding season. The overall purposes of this study were (1) to examine the effects of repeated progestogen priming of prepubertal heifers on the attainment of puberty, (2) to evaluate the potential additive effect of melengestrol acetate (MGA) plus sodium monensin on attainment of puberty in beef heifers, (3) to examine subsequent reproductive performance, including the response to synchronization of estrus, first service conception and overall pregnancy rates and (4) to examine the effects of repeated progestogen priming on follicular dynamics of prepubertal heifers.

In summary, the Rumensin group developed significantly more large follicles (≥ 10 mm in diameter), on average, per animal when compared with the other treatment groups for Replicate 1. For Replicate 2, the control and Rumensin groups developed significantly more medium follicles (6-9 mm in diameter) than either the MGA+Rumensin or MGA groups. During Replicate 2, the MGA group developed significantly more large follicles (≥ 10 mm in diameter) than any of the other treatment groups, but the

MGA+Rumensin and Rumensin groups also developed more large follicles than the control group.

The feeding of sodium monensin significantly increased the fertility of pubertal crossbred beef heifers. The group of animals supplemented with only sodium monensin had a significantly higher pregnancy percentage than either the MGA group or the control group. Though no significant additive effect of MGA was noted for pregnancy percentage, the groups receiving MGA developed numerically greater numbers of corpora lutea prior to the breeding season when compared with the control and Rumensin treatment groups. During Replicate 2, the MGA+Rumensin group was the only group that had significantly greater breeding reproductive tract scores as well as a greater change in reproductive tract scores throughout Replicate 2 of the study when compared with the control animals. This suggests a possible additive effect of MGA when combined with monensin on reproductive tract score.

In conclusion, these experiments provide evidence for the positive effect of sodium monensin on puberty attainment and increased overall pregnancy rates of heifers during their first breeding season when using artificial insemination combined with natural mating. However, the additive effect of melengestrol acetate is non-existent.

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VITA

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